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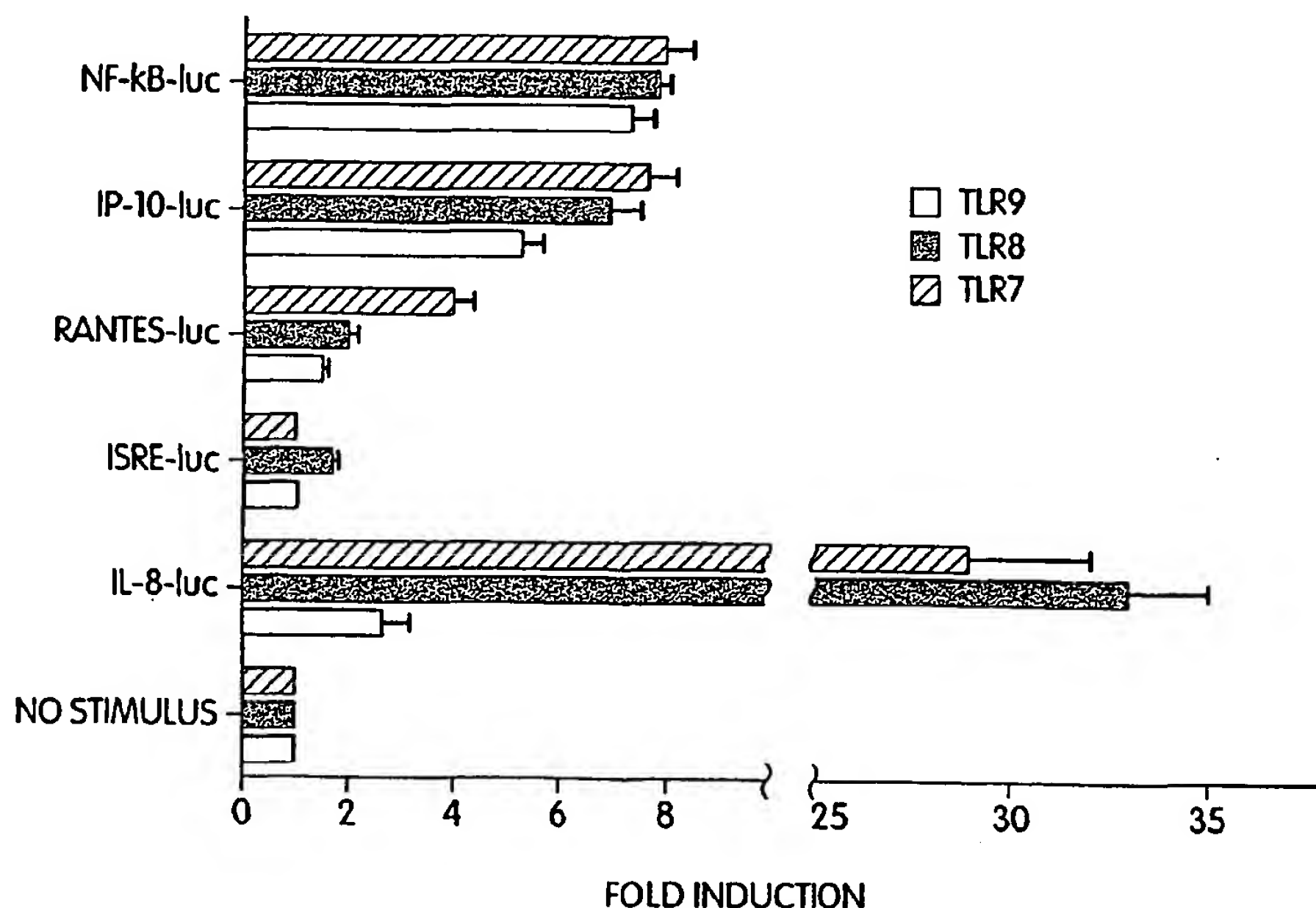
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(54) Title: TOLL-LIKE RECEPTOR 3 SIGNALING AGONISTS AND ANTAGONISTS



(57) Abstract: Compositions and methods are provided to identify, characterize, and optimize immunostimulatory compounds, their agonists and antagonists, working through TLR3.

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TOLL-LIKE RECEPTOR 3 SIGNALING AGONISTS AND ANTAGONISTS**Field of the Invention**

The invention pertains to signal transduction by Toll-like receptor 3 (TLR3),
5 which is believed to be involved in innate immunity. More specifically, the invention
pertains to screening methods useful for the identification and characterization of TLR3
ligands, TLR3 signaling agonists, and TLR3 signaling antagonists.

Background of the Invention

10 Toll-like receptors (TLRs) are a family of at least ten highly conserved receptor
proteins (TLR1 – TLR10) which recognize pathogen-associated molecular patterns
(PAMPs) and act as key elements in innate immunity. As members of the pro-
inflammatory interleukin-1 receptor (IL-1R) family, TLRs share homologies in their
cytoplasmic domains called Toll/IL-1R homology (TIR) domains. PCT published
15 applications PCT/US98/08979 and PCT/US01/16766. Intracellular signaling
mechanisms mediated by TIRs appear generally similar, with MyD88 (Wesche H et al.
(1997) *Immunity* 7:837-47; Medzhitov R et al. (1998) *Mol Cell* 2:253-8; Adachi O et al.
(1998) *Immunity* 9:143-50; Kawai T et al. (1999) *Immunity* 11:115-22) and tumor
necrosis factor receptor-associated factor 6 (TRAF6; Cao Z et al. (1996) *Nature*
20 383:443-6; Lomaga MA et al. (1999) *Genes Dev* 13:1015-24) believed to have critical
roles. Signal transduction between MyD88 and TRAF6 is known to involve members
of the serine-threonine kinase IL-1 receptor-associated kinase (IRAK) family, including
at least IRAK-1 and IRAK-2. Muzio M et al. (1997) *Science* 278:1612-5.

Ligands for many but not all of the TLRs have been described. For instance, it
25 has been reported that TLR2 signals in response to peptidoglycan and lipopeptides.
Yoshimura A et al. (1999) *J Immunol* 163:1-5; Brightbill HD et al. (1999) *Science*
285:732-6; Aliprantis AO et al. (1999) *Science* 285:736-9; Takeuchi O et al. (1999)
Immunity 11:443-51; Underhill DM et al. (1999) *Nature* 401:811-5. TLR4 has been
reported to signal in response to lipopolysaccharide (LPS). Hoshino K et al. (1999) *J*
30 *Immunol* 162:3749-52; Poltorak A et al. (1998) *Science* 282:2085-8; Medzhitov R et al.
(1997) *Nature* 388:394-7. Bacterial flagellin has been reported to be a natural ligand
for TLR5. Hayashi F et al. (2001) *Nature* 410:1099-1103. TLR6, in conjunction with
with TLR2, has been reported to signal in response to proteoglycan. Ozinsky A et al.

(2000) *PNAS USA* 97:13766-71; Takeuchi O et al. (2001) *Int Immunol* 13:933-40.

Recently it was recently reported that TLR9 is a receptor for CpG DNA. Hemmi H et al. (2000) *Nature* 408:740-5.

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Summary of the Invention

The invention provides screening methods and compositions useful for the identification and characterization of compounds which themselves signal through Toll-like receptor 3 (TLR3) or which influence signaling through TLR3. Compounds which themselves signal through TLR3 are presumptively immunostimulatory.

10 Compounds which influence signaling through TLR3 include both agonists and antagonists of TLR3 signaling activity. The methods provided by the invention are adaptable to high throughput screening, thus accelerating the identification and characterization of previously unknown inducers, agonists, and antagonists of TLR3 signaling activity.

15 The methods of the invention rely at least in part on the ability to assess TLR3 signaling activity. It has surprisingly been discovered according to the present invention that reporter constructs having reporter genes under control of certain promoter response elements sensitive to TLR3 signaling activity are useful in the screening assays of the invention. For example it has been surprisingly discovered
20 according to the present invention that a reporter gene under control of interferon-specific response element (ISRE) is sensitive to TLR3 signaling activity.

It has also surprisingly been discovered according to the present invention that screening assays for TLR ligands and other assays involving TLR signaling activity can benefit from optimization for at least one of the variables of (a) concentration of test
25 and/or reference compound, (b) kinetics of the assay, and (c) selection of reporter. Interpretation of assay data can be influenced by each of these variables.

In one aspect the invention provides a screening method for identifying an immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound under
30 conditions which, in absence of the test compound, permit a negative control response mediated by a TLR3 signal transduction pathway; (b) detecting a test response mediated by the TLR3 signal transduction pathway; and (c) determining the test

compound is an immunostimulatory compound when the test response exceeds the negative control response. In this and in all aspects of the invention, in one embodiment the screening method is performed on a plurality of test compounds. A test compound according to this and all aspects of the invention is in one embodiment a member of a library of compounds, preferably a combinatorial library of compounds. Also in this and in all aspects of the invention, a test compound is preferably a small molecule, a nucleic acid, a polypeptide, an oligopeptide, or a lipid. In more preferred embodiments, the test compound is a small molecule or a nucleic acid. In one embodiment a test compound that is a nucleic acid is a CpG nucleic acid.

10 In another aspect the invention provides a screening method for identifying an immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound under conditions which, in presence of a reference immunostimulatory compound, permit a reference response mediated by a TLR3 signal transduction pathway; (b) detecting a test response mediated by the TLR3 signal transduction pathway; and (c) determining the test compound is an immunostimulatory compound when the test response equals or exceeds the reference response. In this and other aspects of the invention, a reference immunostimulatory compound is preferably a small molecule, a nucleic acid, a polypeptide, an oligopeptide, or a lipid. In one embodiment the reference immunostimulatory compound is a CpG nucleic acid.

In a further aspect the invention provides a screening method for identifying a compound that modulates TLR3 signaling activity. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound and a reference immunostimulatory compound under conditions which, in presence of the reference immunostimulatory compound alone, permit a reference response mediated by a TLR3 signal transduction pathway; (b) detecting a test-reference response mediated by the TLR3 signal transduction pathway; (c) determining the test compound is an agonist of TLR3 signaling activity when the test-reference response exceeds the reference response; and (d) determining the test compound is an antagonist of TLR3 signaling activity when the reference response exceeds the test-reference response.

In yet another aspect the invention provides a screening method for identifying species specificity of an immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) measuring a first species-specific response mediated by a TLR3 signal transduction pathway when a functional TLR3 of a first species is contacted with a test compound; (b) measuring a second species-specific response mediated by the TLR3 signal transduction pathway when a functional TLR3 of a second species is contacted with the test compound; and (c) comparing the first species-specific response with the second species-specific response. In a preferred embodiment the functional TLR3 of the first species is a human TLR3. In one preferred embodiment the functional TLR3 of the first species is a human TLR3 and the functional TLR3 of the second species is a mouse TLR3.

In preferred embodiments of the foregoing aspects of the invention, the response mediated by the TLR3 signal transduction pathway is measured quantitatively.

Also in preferred embodiments of the foregoing aspects of the invention, the functional TLR3 is expressed in a cell. For example, in one embodiment the cell is an isolated mammalian cell that naturally expresses the functional TLR3. Alternatively, in another embodiment the cell is an isolated mammalian cell that does not naturally express the functional TLR3, wherein the cell has an expression vector for TLR3. For example, in one preferred embodiment the cell is a human 293 fibroblast. In other embodiments, the functional TLR3 is part of a cell-free system.

Particularly useful in embodiments of the invention involving cells which express functional TLR3 are cells which include a reporter construct sensitive to TLR3 signaling. In one embodiment the cell includes an expression vector having an isolated nucleic acid which encodes a reporter construct selected from the group of nuclear factor-kappa B-luciferase (NF- κ B-luc), IFN-specific response element-luciferase (ISRE-luc), interleukin-6-luciferase (IL-6-luc), interleukin 8-luciferase (IL-8-luc), interleukin 12 p40 subunit-luciferase (IL-12 p40-luc), interleukin 12 p40 subunit-beta galactosidase (IL-12 p40- β -Gal), activator protein 1-luciferase (AP1-luc), interferon alpha-luciferase (IFN- α -luc), interferon beta-luciferase (IFN- β -luc), RANTES-luciferase (RANTES-luc), tumor necrosis factor-luciferase (TNF-luc), IP-10-luciferase

(IP-10-luc), and interferon-inducible T cell alpha chemoattractant-luciferase (I-TAC-luc). In a preferred embodiment the reporter construct is ISRE-luc.

In one embodiment according to each of the foregoing aspects of the invention, the functional TLR3 is part of a complex with a non-TLR protein selected from the group consisting of MyD88, IL-1 receptor associated kinase 1-3 (IRAK1, IRAK2, IRAK3), tumor necrosis factor receptor-associated factor 1-6 (TRAF1 - TRAF6), I κ B, NF- κ B, MyD88-adaptor-like (Mal), Toll-interleukin 1 receptor (TIR) domain-containing adapter protein (TIRAP), Tollip, Rac, and functional homologues and derivatives thereof. In a related embodiment functional TLR3 is part of a complex with a non-TLR protein listed above, excluding MyD88.

Also according to each of the foregoing aspects of the invention, in one embodiment the response mediated by a TLR3 signal transduction pathway is induction of a reporter gene under control of a promoter response element selected from the group consisting of ISRE, IL-6, IL-8, IL-12 p40, IFN- α , IFN- β , IFN- ω , RANTES, TNF, IP-10, and I-TAC. For example, in a preferred embodiment the reporter gene under control of a promoter response element is selected from the group consisting of ISRE-luc, IL-6-luc, IL-8-luc, IL-12 p40-luc, IL-12 p40- β -Gal, IFN- α -luc, IFN- β -luc, RANTES-luc, TNF-luc, IP-10-luc, and I-TAC-luc. In one preferred embodiment the reporter gene under control of a promoter response element is ISRE-luc. In yet another preferred embodiment the reporter gene is selected from the group consisting of IFN- α 1-luc and IFN- α 4-luc.

In yet another embodiment according to each of the foregoing aspects of the invention, the response mediated by a TLR3 signal transduction pathway is selected from the group consisting of (a) induction of a reporter gene under control of a minimal promoter responsive to a transcription factor selected from the group consisting of AP1, NF- κ B, ATF2, IRF3, and IRF7; (b) secretion of a chemokine; and (c) secretion of a cytokine. For example, in one preferred embodiment the response mediated by a TLR3 signal transduction pathway is induction of a reporter gene selected from the group consisting of AP1-luc and NF- κ B-luc. In another preferred embodiment the response mediated by a TLR3 signal transduction pathway is secretion of a type 1 IFN. In yet another preferred embodiment the response mediated by a TLR3 signal transduction

pathway is secretion of a chemokine selected from the group consisting of CCL5 (RANTES), CXCL9 (Mig), CXCL10 (IP-10), and CXCL11 (I-TAC).

The sensitivity and interpretation of the screening methods of the present invention can be optimized. Such optimization involves proper selection of any one or combination of (a) concentration of test and/or reference compound, (b) kinetics of the assay, and (c) reporter. Thus, further according to each of the first three aspects of the invention, in one embodiment the contacting a functional TLR3 with a test compound further entails, for each test compound, contacting with the test compound at each of a plurality of concentrations. For example, each test compound may be evaluated at various concentrations which differ by log increments. Also according to each of the foregoing aspects of the invention, in one embodiment the detecting is performed 4-12 hours, preferably 6-8 hours, following the contacting. Similarly, in yet another embodiment according to each of the foregoing aspects of the invention, the detecting is performed 16-24 hours following the contacting. Detecting performed 4-12 hours, preferably 6-8 hours, following the contacting is believed to be more sensitive to affinity of interaction than is detecting at later times. Detecting performed 16-24 hours or later following the contacting is believed to be more sensitive to stability and duration of receptor/ligand interaction. Furthermore, because certain reporter constructs are more sensitive to certain TLRs than others, proper matching of reporter to TLR assay is important to increase signal-to-noise ratio in the readout of a particular assay.

Brief Description of the Figures

This application includes examples which refer to figures or other drawings. It is to be understood that the referenced figures are illustrative only and are not essential to the enablement of the claimed invention.

Figure 1 is two paired bar graphs showing (A) the induction of NF- κ B and (B) the amount of IL-8 produced by 293 fibroblast cells transfected with human TLR9 in response to exposure to various stimuli, including CpG-ODN, GpC-ODN, LPS, and medium.

Figure 2 is a bar graph showing the induction of NF- κ B produced by 293 fibroblast cells transfected with murine TLR9 in response to exposure to various

stimuli, including CpG-ODN, methylated CpG-ODN (Me-CpG-ODN), GpC-ODN, LPS, and medium.

Figure 3 is a series of gel images depicting the results of reverse transcriptase-polymerase chain reaction (RT-PCR) assays for murine TLR9 (mTLR9), human TLR9
5 (hTLR9), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in untransfected control 293 cells, 293 cells transfected with mTLR9 (293-mTLR9), and 293 cells transfected with hTLR9 (293-hTLR9).

Figure 4 is a graph showing the degree of induction of NF- κ B-luc by various stimuli in stably transfected 293-hTLR9 cells.

10 Figure 5 is a graph showing the degree of induction of NF- κ B-luc by various stimuli in stably transfected 293-mTLR9 cells.

Figure 6 is a graph showing fold induction of response as a function of concentration for a series of four related immunostimulatory nucleic acids contacted with human 293 fibroblast cells stably transfected with murine TLR9 and NF- κ B-luc.
15 Concentrations listed correspond to EC50 for each ligand.

Figure 7 is a graph showing kinetics of EC50 determinations for a series of five immunostimulatory nucleic acids contacted with human 293 fibroblast cells stably transfected with murine TLR9 and NF- κ B-luc.

Figure 8 is a graph showing kinetics of EC50 determinations for the same series
20 of five immunostimulatory nucleic acids as in Figure 7 contacted with human 293 fibroblast cells stably transfected with human TLR9 and NF- κ B-luc.

Figure 9 is a graph showing kinetics of maximal activity (fold induction of response) for the same series of five immunostimulatory nucleic acids as in Figure 7 contacted with human 293 fibroblast cells stably transfected with murine TLR9 and
25 NF- κ B-luc.

Figure 10 is a graph showing kinetics of maximal activity (fold induction of response) for the same series of five immunostimulatory nucleic acids as in Figure 7 contacted with human 293 fibroblast cells stably transfected with human TLR9 and NF- κ B-luc.

30 Figure 11 is a bar graph showing fold induction of response as measured using various luciferase reporter constructs (NF- κ B-luc, IP-10-luc, RANTES-luc, ISRE-luc,

and IL-8-luc) in combination with TLR7, TLR8, and TLR9, each TLR contacted with a specific reference TLR ligand.

Detailed Description of the Invention

5 The invention in certain aspects provides screening methods useful for the identification, characterization, and optimization of immunostimulatory compounds, including but not limited to immunostimulatory nucleic acids and immunostimulatory small molecules, as well as assays for the identification and optimization of agonists and antagonists of TLR3 signaling. The methods according to the invention include
10 both cell-based and cell-free assays. In certain preferred embodiments the screening methods are performed in a high throughput manner. The methods can be used to screen libraries of compounds for their ability to modulate immune activation that involves TLR3 signaling.

 In one aspect the invention provides a screening method for identifying an
15 immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound under conditions which, in absence of the test compound, permit a negative control response mediated by a TLR3 signal transduction pathway; (b) detecting a test response mediated by the TLR3 signal transduction pathway; and (c) determining the test
20 compound is an immunostimulatory compound when the test response exceeds the negative control response. In a second aspect the invention provides a screening method for identifying an immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound under conditions which, in presence of a reference immunostimulatory
25 compound, permit a reference response mediated by a TLR3 signal transduction pathway; (b) detecting a test response mediated by the TLR3 signal transduction pathway; and (c) determining the test compound is an immunostimulatory compound when the test response equals or exceeds the reference response. It will be appreciated that these two aspects of the invention differ in that one involves comparison of the test
30 compound against a negative control and the other involves comparison of the test compound against a positive control.

For these and other aspects of the invention, the TLR3 is preferably a mammalian TLR3, such as human TLR3 or mouse TLR3. Nucleotide and amino acid sequences for human TLR3 and murine TLR3 have previously been described. The nucleotide sequence for human TLR3 cDNA can be found as GenBank accession no. NM_003265 (SEQ ID NO:1), and the deduced amino acid sequence for human TLR3, encompassing 904 amino acids, can be found as GenBank accession nos NP_003256 (SEQ ID NO:2). The nucleotide sequence for murine TLR3 cDNA can be found as GenBank accession no. AF355152 (SEQ ID NO:3), and the deduced amino acid sequence for murine TLR3, encompassing 905 amino acids, can be found as GenBank accession no. AAK26117 (SEQ ID NO:4).

As used herein, a "functional TLR3" shall refer to a polypeptide, including a full length naturally occurring TLR3 polypeptide as described above, which specifically binds a TLR3 ligand and signals via a Toll/interleukin-1 receptor (TIR) domain. In addition to full length naturally occurring TLR3, a functional TLR3 thus also refers to allelic variants, fusion proteins, and truncated versions of the same, provided the polypeptide specifically binds a TLR3 ligand and signals via a TIR domain. In a preferred embodiment, the functional TLR3 includes a human TLR3 extracellular domain having an amino acid sequence provided by amino acids 38-707 according to SEQ ID NO:2. In another preferred embodiment, the functional TLR3 includes a murine TLR3 extracellular domain having an amino acid sequence provided by amino acids 39-708 according to SEQ ID NO:4. Preferably, the functional TLR3 signals through a TIR domain of TLR3.

In certain embodiments of this and other aspects of the invention, the functional TLR3 is expressed, either naturally or artificially, in a cell. In some embodiments, a cell expressing TLR3 for use in the methods of the invention expresses TLR3 and no other TLR. Alternatively, in some embodiments a cell expressing TLR3 for use in the methods of the invention expresses both TLR3 and at least one other TLR, e.g., TLR7, TLR8, or TLR9. In one embodiment the cell is an isolated mammalian cell that naturally expresses functional TLR3. Cells and tissues known to express TLR3 include dendritic cells (DCs), intraepithelial cells, and placenta. Muzio M et al. (2000) *J Immunol* 164:5998-6004; Cario E et al. (2000) *Infect Immun* 68:7010-7; Rock FL et al. (1998) *Proc Natl Acad Sci USA* 95:588-93. The term "isolated" as used herein, with

reference to a cell or to a compound, means substantially free of or separated from components with which the cell or compound is normally associated in nature, e.g., other cells, nucleic acids, proteins, lipids, carbohydrates or *in vivo* systems to an extent practical and appropriate for its intended use.

5 In another embodiment the cell can be one that, as it occurs in nature, is not capable of expressing TLR3 but which is rendered capable of expressing TLR3 through the artificial introduction of an expression vector for TLR3. Examples of cell lines lacking TLR3 include, but are not limited to, human 293 fibroblasts (ATCC CRL-1573) and HEp-2 human epithelial cells (ATCC CCL-23). Examples of cell lines lacking
10 TLR9 include, but are not limited to, human 293 fibroblasts (ATCC CRL-1573), MonoMac-6, THP-1, U937, CHO, and any TLR9 knock-out. Typically the cell, whether it is capable of expressing TLR3 naturally or artificially, preferably has all the necessary elements for signal transduction initiated through the the TLR3 receptor. For example, it is believed that TLR9 signaling requires the adapter protein MyD88 in an
15 early step of signal transduction. In contrast, TLR3 appears not to require MyD88 but may require other factors further downstream, e.g., factors that induce mitogen-activated protein kinase (MAPK) and factors downstream of MAPK.

When indicated, introduction of a particular TLR into a cell or cell line is preferably accomplished by transient or stable transfection of the cell or cell line with a
20 TLR-encoding nucleic acid sequence operatively linked to a gene expression sequence (as described herein). For example, a cell artificially induced to express TLR3 for use in the methods of the invention includes a cell that has been transiently or stably transfected with a TLR3 expression vector. Any suitable method of transient or stable transfection can be employed for this purpose.

25 An expression vector for TLR3 will include at least a nucleotide sequence coding for a functional TLR3 polypeptide, operably linked to a gene expression sequence which can direct the expression of the TLR3 nucleic acid within a eukaryotic or prokaryotic cell. A "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which
30 facilitates the efficient transcription and translation of the nucleic acid to which it is operably linked. With respect to TLR3 nucleic acid, the "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer

combination, which facilitates the efficient transcription and translation of the TLR3 nucleic acid to which it is operably linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the
5 promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, β -actin promoter, and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the simian virus (e.g., SV40), papillomavirus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus (RSV),
10 cytomegalovirus (CMV), the long terminal repeats (LTR) of Moloney murine leukemia virus and other retroviruses, and the thymidine kinase (TK) promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent.
15 For example, the metallothionein (MT) promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

In general, the gene expression sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of
20 transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined TLR3 nucleic acid. The gene expression sequences optionally include enhancer sequences or upstream activator sequences as desired.

25 Generally a nucleic acid coding sequence and a gene expression sequence are said to be "operably linked" when they are covalently linked in such a way as to place the transcription and/or translation of the nucleic acid coding sequence under the influence or control of the gene expression sequence. Thus the TLR3 nucleic acid sequence and the gene expression sequence are said to be "operably linked" when they
30 are covalently linked in such a way as to place the transcription and/or translation of the TLR3 coding sequence under the influence or control of the gene expression sequence. If it is desired that the TLR3 sequence be translated into a functional protein, two DNA

sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the TLR3 sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the TLR3 sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to a TLR3 nucleic acid sequence if the gene expression sequence were capable of effecting transcription of that TLR3 nucleic acid sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

In certain embodiments a TLR expression vector is constructed so as to permit tandem expression of two distinct TLRs, e.g., both TLR3 and a second TLR. Such a tandem expression vector can be used when it is desired to express two TLRs using a single transformation or transfection. Alternatively, a TLR3 expression vector can be used in conjunction with a second expression vector constructed so as to permit expression of a second TLR.

The screening assays can have any of a number of possible readout systems based upon a TLR/IL-1R signal transduction pathway. In preferred embodiments, the readout for the screening assay is based on the use of native genes or, alternatively, transfected or otherwise artificially introduced reporter gene constructs which are responsive to the TLR/IL-1R signal transduction pathway involving MyD88, TRAF, p38, and/or ERK. Häcker H et al. (1999) *EMBO J* 18:6973-82. These pathways activate kinases including κ B kinase complex and c-Jun N-terminal kinases. Thus reporter genes and reporter gene constructs particularly useful for the assays include, e.g., a reporter gene operatively linked to a promoter sensitive to NF- κ B. Examples of such promoters include, without limitation, those for NF- κ B, IL-1 β , IL-6, IL-8, IL-12 p40, CD80, CD86, and TNF- α . The reporter gene operatively linked to the TLR-sensitive promoter can include, without limitation, an enzyme (e.g., luciferase, alkaline phosphatase, β -galactosidase, chloramphenicol acetyltransferase (CAT), etc.), a bioluminescence marker (e.g., green-fluorescent protein (GFP, U.S. patent 5,491,084), etc.), a surface-expressed molecule (e.g., CD25), and a secreted molecule (e.g., IL-8, IL-12 p40, TNF- α). In certain preferred embodiments the reporter is selected from IL-

8, TNF- α , NF- κ B-luciferase (NF- κ B-luc; Häcker H et al. (1999) *EMBO J* 18:6973-82), IL-12 p40-luc (Murphy TL et al. (1995) *Mol Cell Biol* 15:5258-67), and TNF-luc (Häcker H et al. (1999) *EMBO J* 18:6973-82). In assays relying on enzyme activity readout, substrate can be supplied as part of the assay, and detection can involve
5 measurement of chemiluminescence, fluorescence, color development, incorporation of radioactive label, drug resistance, or other marker of enzyme activity. For assays relying on surface expression of a molecule, detection can be accomplished using flow cytometry (FACS) analysis or functional assays. Secreted molecules can be assayed using enzyme-linked immunosorbent assay (ELISA) or bioassays. These and other
10 suitable readout systems are well known in the art and are commercially available.

Thus a cell expressing a functional TLR3 and useful for the methods of the invention has, in some embodiments, an expression vector comprising an isolated nucleic acid which encodes a reporter construct useful for detecting TLR signaling. The expression vector comprising an isolated nucleic acid which encodes a reporter
15 construct useful for detecting TLR signaling can include a reporter gene under control of a minimal promoter responsive to a transcription factor believed by the applicant to be activated as a consequence of TLR3 signaling. Examples of such minimal promoters include, without limitation, promoters for the following genes: AP1, NF- κ B, ATF2, IRF3, and IRF7. In other embodiments the expression vector comprising an
20 isolated nucleic acid which encodes a reporter construct useful for detecting TLR signaling can include a gene under control of a promoter response element selected from IL-6, IL-8, IL-12 p40 subunit, a type 1 IFN, RANTES, TNF, IP-10, I-TAC, and ISRE. The promoter response element generally will be present in multiple copies, e.g., as tandem repeats. For example, an ISRE-luciferase reporter construct useful in
25 the invention is available from Stratagene (catalog no. 219092) and includes a 5x ISRE tandem repeat joined to a TATA box upstream of a luciferase reporter gene. As discussed further elsewhere herein, the reporter itself can be any gene product suitable for detection by methods recognized in the art. Such methods for detection can include, for example, measurement of spontaneous or stimulated light emission, enzyme
30 activity, expression of a soluble molecule, expression of a cell surface molecule, etc.

As mentioned above, the functional TLR3 is contacted with a test compound in order to identify an immunostimulatory compound. An immunostimulatory compound

is a natural or synthetic compound that is capable of inducing an immune response when contacted with an immune cell. In the context of the methods of the invention, an immunostimulatory compound refers to a natural or synthetic compound that is capable of inducing an immune response when contacted with an immune cell expressing a functional TLR3 polypeptide. Preferably the immune response is or involves activation of a TLR3 signal transduction pathway. Thus immunostimulatory compounds identified and characterized using the methods of the invention specifically include TLR3 ligands, i.e., compounds which selectively bind to TLR3 and induce a TLR3 signal transduction pathway. Immunostimulatory compounds in general include but are not limited to nucleic acids, including oligonucleotides and polynucleotides; oligopeptides; polypeptides; lipids, including lipopolysaccharides; carbohydrates, including oligosaccharides and polysaccharides; and small molecules. Accordingly, a "test compound" refers to nucleic acids, including oligonucleotides and polynucleotides; oligopeptides; polypeptides; lipids, including lipopolysaccharides; carbohydrates, including oligosaccharides and polysaccharides; and small molecules. Test compounds include compounds with known biological activity as well as compounds without known biological activity.

A "reference immunostimulatory compound" refers to an immunostimulatory compound that characteristically induces an immune response when contacted with an immune cell expressing a functional TLR polypeptide. In the screening methods of the invention, the reference immunostimulatory compound is a natural or synthetic compound that characteristically induces an immune response when contacted with an immune cell expressing a functional TLR3 polypeptide. Preferably the immune response is or involves activation of a TLR3 signal transduction pathway. Thus a reference immunostimulatory compound will characteristically induce a reference response mediated by a TLR3 signal transduction pathway when contacted with a functional TLR3 under suitable conditions. The reference response can be measured according to any of the methods described herein. Importantly, a reference immunostimulatory compound specifically includes a test compound identified as an immunostimulatory compound according to any one of the methods of the invention. Therefore a reference immunostimulatory compound can be a nucleic acid, including oligonucleotides and polynucleotides; an oligopeptide; a polypeptide; a lipid, including

lipopolysaccharides; a carbohydrate, including oligosaccharides and polysaccharides; or a small molecule.

Small molecules include naturally occurring, synthetic, and semisynthetic organic and organometallic compounds with molecular weight less than about 1.5 kDa.

5 Examples of small molecules include most drugs, subunits of polymeric materials, and analogs and derivatives thereof.

A "nucleic acid" as used herein with respect to test compounds and reference compounds used in the methods of the invention, shall refer to any polymer of two or more individual nucleoside or nucleotide units. Typically individual nucleoside or
10 nucleotide units will include any one or combination of deoxyribonucleosides, ribonucleosides, deoxyribonucleotides, and ribonucleotides. The individual nucleotide or nucleoside units of the nucleic acid can be naturally occurring or not naturally occurring. For example, the individual nucleotide units can include deoxyadenosine, deoxycytidine, deoxyguanosine, thymidine, and uracil. In addition to naturally
15 occurring 2'-deoxy and 2'-hydroxyl forms, individual nucleosides also include synthetic nucleosides having modified base moieties and/or modified sugar moieties, e.g., as described in Uhlmann E et al. (1990) *Chem Rev* 90:543-84. The linkages between individual nucleotide or nucleoside units can be naturally occurring or not naturally occurring. For example, the linkages can be phosphodiester, phosphorothioate,
20 phosphorodithioate, phosphoramidate, as well as peptide linkages and other covalent linkages, known in the art, suitable for joining adjacent nucleoside or nucleotide units. The nucleic acid test compounds and nucleic acid reference compounds typically range in size from 3-4 units to a few tens of units, e.g., 18-40 units.

The substituted purines and pyrimidines of the ISNAs include standard purines
25 and pyrimidines such as cytosine as well as base analogs such as C-5 propyne substituted bases. Wagner RW et al. (1996) *Nat Biotechnol* 14:840-4. Purines and pyrimidines include but are not limited to adenine, cytosine, guanine, thymine, 5-methylcytosine, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, and other naturally and non-naturally occurring nucleobases, substituted
30 and unsubstituted aromatic moieties.

Libraries of compounds that can be used as test compounds are available from various commercial suppliers, and they can be made to order using techniques well

known in the art, including combinatorial chemistry techniques. Especially in combination with high throughput screening methods, such methods including in particular automated multichannel methods of screening, large libraries of test compounds can be screened according to the methods of the invention. Large libraries
5 can include hundreds, thousands, tens of thousands, hundreds of thousands, and even millions of compounds.

Thus in preferred embodiments, the methods for screening test compounds can be performed on a large scale and with high throughput by incorporating, e.g., an array-based assay system and at least one automated or semi-automated step. For example,
10 the assays can be set up using multiple-well plates in which cells are dispensed in individual wells and reagents are added in a systematic manner using a multiwell delivery device suited to the geometry of the multiwell plate. Manual and robotic multiwell delivery devices suitable for use in a high throughput screening assay are well known by those skilled in the art. Each well or array element can be mapped in a
15 one-to-one manner to a particular test condition, such as the test compound. Readouts can also be performed in this multiwell array, preferably using a multiwell plate reader device or the like. Examples of such devices are well known in the art and are available through commercial sources. Sample and reagent handling can be automated to further enhance the throughput capacity of the screening assay, such that dozens,
20 hundreds, thousands, or even millions of parallel assays can be performed in a day or in a week. Fully robotic systems are known in the art for applications such as generation and analysis of combinatorial libraries of synthetic compounds. See, for example, U.S. patents 5,443,791 and 5,708,158.

A "CpG nucleic acid" or a "CpG immunostimulatory nucleic acid" as used
25 herein is a nucleic acid containing at least one unmethylated CpG dinucleotide (cytosine-guanine dinucleotide sequence, i.e. "CpG DNA" or DNA containing a 5' cytosine followed by 3' guanine and linked by a phosphate bond) and activates a component of the immune system. The entire CpG nucleic acid can be unmethylated or portions may be unmethylated but at least the C of the 5' CG 3' must be unmethylated.

30 In one embodiment a CpG nucleic acid is represented by at least the formula:



wherein X_1 and X_2 are nucleotides, N is any nucleotide, and N_1 and N_2 are nucleic acid sequences composed of from about 0-25 N's each. In some embodiments X_1 is adenine, guanine, or thymine and/or X_2 is cytosine, adenine, or thymine. In other embodiments X_1 is cytosine and/or X_2 is guanine.

- 5 Examples of CpG nucleic acids according to the invention include but are not limited to those listed in Table 1.

Table 1. Exemplary CpG Nucleic Acids

	AACGTTCT	
10	AAGCGAAAATGAAATTGACT	SEQ ID NO:39
	ACCATGGACGAACTGTTTCCCCTC	SEQ ID NO:40
	ACCATGGACGACCTGTTTCCCCTC	SEQ ID NO:41
	ACCATGGACGAGCTGTTTCCCCTC	SEQ ID NO:42
	ACCATGGACGATCTGTTTCCCCTC	SEQ ID NO:43
15	ACCATGGACGGTCTGTTTCCCCTC	SEQ ID NO:44
	ACCATGGACGTACTGTTTCCCCTC	SEQ ID NO:45
	ACCATGGACGTTCTGTTTCCCCTC	SEQ ID NO:46
	AGCGGGGGCGAGCGGGGGCG	SEQ ID NO:47
	AGCTATGACGTTCCAAGG	SEQ ID NO:48
20	ATCGACTCTCGAGCGTTCTC	SEQ ID NO:49
	ATGACGTTCTGACGTT	SEQ ID NO:50
	ATGGAAGGTCCAACGTTCTC	SEQ ID NO:51
	ATGGAAGGTCCAGCGTTCTC	SEQ ID NO:52
	ATGGACTCTCCAGCGTTCTC	SEQ ID NO:53
25	ATGGAGGCTCCATCGTTCTC	SEQ ID NO:54
	CAACGTT	
	CACGTTGAGGGGCAT	SEQ ID NO:55
	CAGGCATAACGTTCCGTAG	SEQ ID NO:56
	CCAACGTT	
30	CTGATTTCCCCGAAATGATG	SEQ ID NO:57
	GAGAACGATGGACCTTCCAT	SEQ ID NO:58
	GAGAACGCTCCAGCACTGAT	SEQ ID NO:59
	GAGAACGCTCGACCTTCCAT	SEQ ID NO:60
	GAGAACGCTCGACCTTCGAT	SEQ ID NO:61
35	GAGAACGCTGGACCTTCCAT	SEQ ID NO:62
	GATTGCCTGACGTCAGAGAG	SEQ ID NO:63
	GCATGACGTTGAGCT	SEQ ID NO:64
	GCGGCGGGCGGCGCGCGCCC	SEQ ID NO:65
	GCGTGCGTTGTCGTTGTCGTT	SEQ ID NO:66
40	GCTAGACGTTAGCGT	SEQ ID NO:67
	GCTAGACGTTAGTGT	SEQ ID NO:68
	GCTAGATGTTAGCGT	SEQ ID NO:69
	GCTTGATGACTCAGCCGGAA	SEQ ID NO:70
	GGAATGACGTTCCCTGTG	SEQ ID NO:71

	GGGGTCAACGTTGACGGGG	SEQ ID NO:72
	GGGGTCAGTCTTGACGGGG	SEQ ID NO:73
	GTCCATTTCCCGTAAATCTT	SEQ ID NO:74
	GTCGCT	
5	GTCGTT	
	TACCGCGTGCGACCCTCT	SEQ ID NO:75
	TCAACGTC	
	TCAACGTT	
	TCAGCGCT	
10	TCAGCGTGCGCC	SEQ ID NO:76
	TCATCGAT	
	TCCACGACGTTTTCGACGTT	SEQ ID NO:77
	TCCATAACGTTTCCTGATGCT	SEQ ID NO:78
	TCCATAGCGTTCCTAGCGTT	SEQ ID NO:79
15	TCCATCACGTGCCTGATGCT	SEQ ID NO:80
	TCCATGACGGTCCTGATGCT	SEQ ID NO:81
	TCCATGACGTCCCTGATGCT	SEQ ID NO:82
	TCCATGACGTGCCTGATGCT	SEQ ID NO:83
	TCCATGACGTTTCCTGACGTT	SEQ ID NO:84
20	TCCATGACGTTTCCTGATGCT	SEQ ID NO:18
	TCCATGCCGGTCCTGATGCT	SEQ ID NO:85
	TCCATGCGTGCGTGCGTTTTT	SEQ ID NO:86
	TCCATGCGTTGCGTTGCGTT	SEQ ID NO:87
	TCCATGGCGGTCCTGATGCT	SEQ ID NO:88
25	TCCATGTCGATCCTGATGCT	SEQ ID NO:89
	TCCATGTCGCTCCTGATGCT	SEQ ID NO:90
	TCCATGTCGGTCCTGATGCT	SEQ ID NO:91
	TCCATGTCGGTCCTGCTGAT	SEQ ID NO:92
	TCCATGTCGTCCCTGATGCT	SEQ ID NO:93
30	TCCATGTCGTTTCCTGATGCT	SEQ ID NO:94
	TCCATGTCGTTTCCTGTGCGTT	SEQ ID NO:95
	TCCATGTCGTTTTTTGTCGTT	SEQ ID NO:96
	TCCTGACGTTTCCTGACGTT	SEQ ID NO:97
	TCCTGTCGTTTCCTGTGCGTT	SEQ ID NO:98
35	TCCTGTCGTTTCCTTGTCGTT	SEQ ID NO:99
	TCCTGTCGTTTTTTTGTCGTT	SEQ ID NO:100
	TCCTTGTCGTTTCCTGTGCGTT	SEQ ID NO:101
	TCGATCGGGGCGGGGCGAGC	SEQ ID NO:102
	TCGTGCTGTCTCCGCTTCTT	SEQ ID NO:103
40	TCGTGCTGTCTCCGCTTCTTCTTGCC	SEQ ID NO:104
	TCGTGCTGTCTGCCCTTCTT	SEQ ID NO:105
	TCGTGCTGTTGTGCGTTTCTT	SEQ ID NO:106
	TCGTGCTGTCGTT	SEQ ID NO:107
	TCGTGCTTGTCGTTTGTCGTT	SEQ ID NO:108
45	TCGTGCTTGTCGTTTTTGTCGTT	SEQ ID NO:109
	TCGTGCTTTTTGTCGTTTTGTCGTT	SEQ ID NO:15
	TCTCCCAGCGCGCGCCAT	SEQ ID NO:110
	TCTCCCAGCGGGCGCAT	SEQ ID NO:111

	TCTCCCAGCGTGCGCCAT	SEQ ID NO:112
	TCTTCGAA	
	TGCAGATTGCGCAATCTGCA	SEQ ID NO:113
	TGTCGCT	
5	TGTCGTT	
	TGTCGTTGTCGTT	SEQ ID NO:114
	TGTCGTTGTCGTTGTCGTT	SEQ ID NO:115
	TGTCGTTGTCGTTGTCGTTGTCGTT	SEQ ID NO:116
	TGTCGTTTGTGTTGTCGTT	SEQ ID NO:117
10		

As used herein the term "response mediated by a TLR signal transduction pathway" refers to a response which is characteristic of an interaction between a TLR and an immunostimulatory compound that induces signaling events through the TLR. Such responses typically involve usual elements of Toll/IL-1R signaling, e.g., MyD88, TRAF, and IRAK molecules, although in the case of TLR3 the role of MyD88 is less clear than for other TLR family members. As demonstrated herein such responses include the induction of a gene under control of a specific promoter such as a NF- κ B promoter, increases in particular cytokine levels, increases in particular chemokine levels etc. The gene under the control of the NF- κ B promoter may be a gene which naturally includes an NF- κ B promoter or it may be a gene in a construct in which an NF- κ B promoter has been inserted. Genes which naturally include the NF- κ B promoter include but are not limited to IL-8, IL-12 p40, NF- κ B-luc, IL-12 p40-luc, and TNF-luc. Increases in cytokine levels may result from increased production or increased stability or increased secretion of the cytokines in response to the TLR-immunostimulatory compound interaction. Th1 cytokines include but are not limited to IL-2, IFN- γ , and IL-12. It has unexpectedly been discovered, according to the instant invention, that the promoter response element ISRE is directly activated as a result of signaling through the TLR3 signal transduction pathway, i.e., independent of IFN- γ production. Th2 cytokines include but are not limited to IL-4, IL-5, and IL-10. Chemokines of particular significance in the invention include but are not limited to CCL5 (RANTES), CXCL9 (Mig), CXCL10 (IP-10), and CXCL11 (I-TAC).

In another aspect the invention provides a screening method for identifying a compound that modulates TLR3 signaling activity. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound and a reference immunostimulatory compound under conditions which,

in presence of the reference immunostimulatory compound alone, permit a reference response mediated by a TLR3 signal transduction pathway; (b) detecting a test-reference response mediated by the TLR3 signal transduction pathway; (c) determining the test compound is an agonist of TLR3 signaling activity when the test-reference response exceeds the reference response; and (d) determining the test compound is an antagonist of TLR3 signaling activity when the reference response exceeds the test-reference response. A test-reference response refers to a type of test response as determined when a test compound and a reference immunostimulatory compound are simultaneously contacted with the TLR3. When a test compound is neither an agonist nor an antagonist of TLR3 signaling activity, the test-reference response and the reference response are indistinguishable.

An agonist as used herein is a compound which causes an enhanced response of a TLR to a reference stimulus. The enhanced response can be additive or synergistic with respect to the response to the reference stimulus by itself. Furthermore, an agonist can work directly or indirectly to cause the enhanced response. Thus an agonist of TLR3 signaling activity as used herein is a compound which causes an enhanced response of a TLR to a reference stimulus.

An antagonist as used herein is a compound which causes a diminished response of a TLR to a reference stimulus. Furthermore, an antagonist can work directly or indirectly to cause the diminished response. Thus an antagonist of TLR3 signaling activity as used herein is a compound which causes a diminished response of a TLR to a reference stimulus.

In addition to identification and characterization of immunostimulatory compounds, agonists of TLR3 signaling, and antagonists of TLR3 signaling, the methods of the invention also permit optimization of lead compounds. Optimization of a lead compound involves an iterative application of a screening method of the invention, further including the steps of selecting the best candidate at any given stage or round in the screening and then substituting it as a benchmark or reference in a subsequent round of screening. This latter process can further include selection of parameters to modify in choosing and generating candidate test compounds to screen. For example, a lead compound from a particular round of screening can be used as a

basis to develop a focused library of new test compounds for use in a subsequent round of screening.

In another aspect the invention provides a screening method for identifying species specificity of an immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) measuring a first species-specific response mediated by a TLR3 signal transduction pathway when a functional TLR3 of a first species is contacted with a test compound; (b) measuring a second species-specific response mediated by the TLR3 signal transduction pathway when a functional TLR3 of a second species is contacted with the test compound; and (c) comparing the first species-specific response with the second species-specific response.

A species-specific TLR, including TLR3, is not limited to a human TLR, but rather can include a TLR derived from human or non-human sources. Examples of non-human sources include, but are not limited to, murine, rat, bovine, canine, feline, ovine, porcine, and equine. Other species include chicken and fish, e.g., aquaculture species.

The species-specific TLR, including TLR3, also is not limited to native TLR polypeptides. In certain embodiments the TLR can be, e.g., a chimeric TLR in which the extracellular domain and the cytoplasmic domain are derived from TLR polypeptides from different species. Such chimeric TLR polypeptides, as described above, can include, for example, a human TLR extracellular domain and a murine TLR cytoplasmic domain, each domain derived from the corresponding TLR of each species. In alternative embodiments, such chimeric TLR polypeptides can include chimeras created with different TLR splice variants or allotypes. Other chimeric TLR polypeptides useful for the screening methods of the invention include chimeric polypeptides created with a TLR of a first type, e.g., TLR3, and another TLR, e.g., TLR7, TLR8, or TLR9, of the same or another species as the TLR of the first type. Also contemplated are chimeric polypeptides which incorporate sequences derived from more than two polypeptides, e.g., an extracellular domain, a transmembrane domain, and a cytoplasmic domain all derived from different polypeptide sources, provided at least one such domain derives from a TLR3 polypeptide. As a further example, also contemplated are constructs such as include an extracellular domain of one TLR3, an intracellular domain of another TLR3, and a non-TLR reporter such as

luciferase, GFP, etc. Those of skill in the art will recognize how to design and generate DNA sequences coding for such chimeric TLR polypeptides.

It has also been discovered, according to the instant invention, that TLR-based screening assays, including but not limited to the TLR3-based assays described herein, are sensitive to parameters such as concentration of test compound, stability of test compound, kinetics of detection, and selection of reporter. These parameters can be optimized in order to derive the most information from a given screening assay. Importantly, the kinetics of detection appear to afford separation of types of information such as affinity of interaction and stability or duration of interaction. For example, measurements taken at earlier timepoints, e.g., after 6-8 hours of contact between TLR and test and/or reference compound, appear to reflect more information about affinity of interaction than do measurements obtained at later timepoints, e.g., after 16-24 or more hours of contact. In addition, while NF- κ B-driven reporters are generally useful in TLR-based screening assays like those of the instant invention, in some instances a reporter other than an NF- κ B-driven reporter will afford greater sensitivity. For example, the IL-8-luc reporter is significantly more sensitive to TLR7 and TLR8 than NF- κ B-luc. Selection of reporter thus appears to be TLR-dependent, while parameters relating to kinetics and concentration appear to be more compound-dependent. Thus in performing the screening methods of the instant invention, it is expected that the methods will be enhanced by inclusion of measurements obtained using at least two concentrations and two time points for each test compound. Typically at least three concentrations will be employed, spanning a two to three log-fold range of concentrations. Finer ranges of concentration can of course be employed under suitable circumstances, for instance based on results of an earlier screening performed using a wider initial range of concentrations.

The invention will be more fully understood by reference to the following examples. These examples, however, are merely intended to illustrate certain embodiments of the invention and are not to be construed to limit the scope of the invention.

Examples

Example 1. Expression Vectors for Human TLR3 (hTLR3) and Murine TLR3 (mTLR3)

To create an expression vector for human TLR3, human TLR3 cDNA was amplified by the polymerase chain method (PCR) from a cDNA made from human 293 cells using the primers

5'-GAAACTCGAGCCACCATGAGACAGACTTTGCCTTGTATCTAC-3' (sense, SEQ ID NO:9) and 5'-GAAAGAATTCTTAATGTACAGAGTTTTTGGATCCAAG-3' (antisense, SEQ ID NO:10). The primers introduce Xho I and EcoRI restriction endonuclease sites at their 5' ends for use in subsequent cloning into the expression vector. The resulting amplication product fragment was cloned into pGEM-T Easy vector (Promega), isolated, cut with Xho I and EcoRI restriction endonucleases, ligated into an Xho I/EcoRI-digested pcDNA3.1 expression vector (Invitrogen). The insert was fully sequenced and translated into protein. The cDNA sequence corresponds to the published cDNA sequence for hTLR3, available as GenBank accession no. NM_003265 (SEQ ID NO:1). The open reading frame codes for a protein 904 amino acids long, having the sequence corresponding to GenBank accession no. NP_003256 (SEQ ID NO:2).

Table 2. cDNA Sequence for Human TLR3

(GenBank Accession No. NM_003265; SEQ ID NO:1)

g	c	g	g	c	c	g	c	g	t	c	g	a	c	a	a	t	g	t	c	t	g	a	t	t	g		60
a	t	c	c	a	a	c	a	g	a		a	t	c	a	t	g	a	g	a	c		a	g	a	c	t	120
c	t	t	t	g	g	a	t	g		c	t	c	c	a	c	c	a	c	a		c	a	a	g	t	g	180
c	t	g	c	a	g	c	c	a	c		c	t	g	a	a	g	t	t	g	a		c	t	c	a	g	240
g	a	a	c	c	t	t	a	c		c	a	t	a	a	c	a	c		t	c	a	a	g	a	t	t	300
g	c	t	a	a	c	t	a	g		t	g	g	a	t	g	t	a		g	a	t	t	a	a	c	a	360
g	a	a	c	t	t	c	c		a	t	g	t	a	a	a	a	g		t	t	t	g	a	a	c	t	420
t	a	a	a	c	c	t	t		g	c	c	t	t	c	t	g	c		a	g	a	t	t	t	g	a	480
g	a	a	a	t	t	a	a		a	a	t	a	a	c	c	c	t		t	t	g	t	c	a	a	g	540
t	a	a	t	g	g	c	t	t		t	c	a	t	c	t	a	c	a		a	a	t	t	a	g	a	600
t	c	t	a	t	t	a	t	c	a		a	a	c	a	a	t	a	a	a		a	a	a	a	g	t	660
t	t	c	a	t	c	t	t	t	a		a	a	a	a	a	t	t	a	g		a	g	t	t	g	t	720
t	t	t	t	c	a	g	c	a		a	t	t	g	a	a	g	a	t		t	a	t	t	g	c	c	780
c	c	t	t	a	c	a	g	a		a	a	g	c	t	a	t	g	t		t	g	a	a	t	t	a	840
t	a	a	c	a	g	c	c	a		c	t	g	t	c	c	a	c	a		a	a	c	t	t	t	c	900
c	a	c	t	a	t	g	c	t		g	a	t	c	t	t	t	c	c		a	a	c	a	a	c	t	960
g	c	t	t	c	c	a	c	a		c	t	a	g	a	a	t	a	t		t	c	t	t	c	t	a	1020
c	t	c	t	t	t	g	c	a		g	g	g	c	t	t	t	t	c	a		a	t	g	t	g	a	1080
a	a	g	t	a	t	t	t	c		c	t	t	g	c	c	t	c	a		t	c	c	c	a	a	g	1140
t	t	t	g	g	a	g	c	a		c	t	t	a	a	c	a	t	g		a	a	g	a	t	t	a	1200
a	g	g	a	t	t	g	a		a	a	c	c	t	g	a	a	t		a	c	t	t	a	a	g	t	1260
g	a	c	a	a	t	g	a		a	c	a	t	t	g	t	a	t		c	a	c	t	t	g	c	a	1320
g	a	a	t	a	a	a	t	c		t	c	a	a	a	a	t	a	g		a	g	a	t	g	a	t	1380

	tgacctgggc	cttaatgaaa	ttgggcaaga	actcacaggc	caggaatgga	gaggtctaga	1440
	aaatatatttc	gaaatctatc	tttcctacaa	caagtacctg	cagctgacta	ggaactcctt	1500
	tgccttggtc	ccaagccttc	aacgactgat	gctccgaagg	gtggccctta	aaaatgtgga	1560
	tagctctcct	tcaccattcc	agcctcttcg	taacttgacc	attctggatc	taagcaacaa	1620
5	caacatagcc	aacataaatg	atgacatggt	ggaggggtctt	gagaaactag	aaattctcga	1680
	tttgcagcat	aacaacttag	cacggctctg	gaaacacgca	aaccctgggtg	gtcccattta	1740
	tttcctaaag	ggtctgtctc	acctccacat	ccttaacttg	gagtccaacg	gctttgacga	1800
	gatcccagtt	gaggtcttca	aggattttatt	tgaactaaag	atcatcgatt	taggattgaa	1860
	taattttaaac	acacttccag	catctgtctt	taataatcag	gtgtctctaa	agtcattgaa	1920
10	ccttcagaag	aatctcataa	catccgttga	gaagaagggt	ttcggggccag	ctttcaggaa	1980
	cctgactgag	ttagatatgc	gctttaatcc	ctttgattgc	acgtgtgaaa	gtattgcctg	2040
	gtttgttaat	tggattaacg	agaccatac	caacatccct	gagctgtcaa	gccactacct	2100
	ttgcaacact	ccacctcact	atcatgggtt	cccagtgaga	cttttttgata	catcatcttg	2160
	caaagacagt	gccccctttg	aactcttttt	catgatcaat	accagtatcc	tggtgatttt	2220
15	tatctttatt	gtacttctca	tccactttga	gggctggagg	atatcttttt	attggaatgt	2280
	ttcagtacat	cgagttcttg	gtttcaaaga	aatagacaga	cagacagaac	agtttgaata	2340
	tgcagcatat	ataattcatg	cctataaaga	taaggattgg	gtctgggaac	atttctcttc	2400
	aatggaaaag	gaagaccaat	ctctcaaatt	ttgtctggaa	gaaagggact	ttgaggcggg	2460
	tgtttttgaa	ctagaagcaa	ttgttaacag	catcaaaaaga	agcagaaaaa	ttatttttgt	2520
20	tataacacac	catctattaa	aagaccatt	atgcaaaaaga	ttcaaggtag	atcatgcagt	2580
	tcaacaagct	attgaacaaa	atctggattc	cattatattg	gttttccttg	aggagattcc	2640
	agattataaa	ctgaaccatg	cactctgttt	gcgaagagga	atgtttaaat	ctcactgcat	2700
	cttgaactgg	ccagttcaga	aagaacggat	aggtgccttt	cgtcataaat	tgcaagtagc	2760
	acttgatcc	aaaaactctg	tacattaaat	ttattttaa	attcaattag	caaaggagaa	2820
25	actttctcaa	tttaaaaagt	tctatggcaa	atttaagttt	tccataaagg	tggtataatt	2880
	tgtttattca	tatttgtaaa	tgattatatt	ctatcacaat	tacatctctt	ctaggaaaat	2940
	gtgtctcctt	atttcaggcc	tatttttgac	aattgactta	attttaccca	aaataaaaaca	3000
	tataagcacg	caaaaaaaaa	aaaaaaaaa				3029

30 Table 3. Amino Acid Sequence for Human TLR3

(GenBank Accession No. NP_003256; SEQ ID NO:2)

	MRQTLPCIYF	WGGLLPFGML	CASSTTKCTV	SHEVADCSHL	KLTQVPDDL	TNITVLNLTH	60
	NQLRRLPAAN	FTRYSQLTSL	DVGFNITISK	EPELCQKLPM	LKVLNLQHNE	LSQLSDKTFA	120
	FCTNLTELHL	MSNSIQKIKN	NPFVKQKNLI	TLDLSHNGLS	STKLGTQVQL	ENLQELLSN	180
35	NKIQALKSEE	LDIFANSSLK	KLELSSNQIK	EFSPGCFHAI	GRLFGFLN	VQLGPSLTEK	240
	LCLELANTSI	RNLSLSNSQL	STTSNTTFLG	LKWTNLTMLD	LSYNNLNVVG	NDSFAWLPQL	300
	EYFFLEYNNI	QHLFSLHSG	LFNVRYLNK	RSFTKQSI	ASLPKIDDFS	FQWLKCLEHL	360
	NMEDNDIPGI	KSNMFTGLIN	LKYLSSNSF	TSLRTLNET	FVSLAHSPLH	ILNLTKNKIS	420
	KIESDAFSWL	GHLEVLDLGL	NEIGQELTGO	EWRGLENIFE	IYLSYNKYLO	LTRNSFALVP	480
40	SLQRLMLRRV	ALKNVDS	PFQPLRNLT	LDLSNNNI	INDDMLEGLE	KLEILDLOHN	540
	NLARLWKHAN	PGGPIYFLKG	LSHLHILNLE	SNGFDEIPVE	VFKDLFELKI	IDLGLNNLNT	600
	LPASVFNNQV	SLKSLNLQKN	LITSVEKKVF	GPAFRNLTEL	DMRFNPFDC	CESIAWFVNW	660
	INETHTNIPE	LSSHLYLCNTP	PHYHGFPVRL	FDTSSCKDSA	PFELFFMINT	SILLIFIFIV	720
	LLIHFEGWRI	SFYWNVSVHR	VLGFKEIDRQ	TEQFEYAAYI	IHAYKDKDWV	WEHFSSMEKE	780
45	DQSLKFCLEE	RDFAAGVFEL	EAIVNSIKRS	RKIIFVITHH	LLKDPLCKRF	KVHHAVQQAI	840
	EQNLDSIILV	FLEEIPDYKL	NHALCLRRGM	FKSHCILNWP	VQKERIGAFR	HKLQVALGSK	900
	NSVH						904

Corresponding nucleotide and amino acid sequences for murine TLR3

50 (mTLR3) are known. The nucleotide sequence of mTLR3 cDNA has been reported as GenBank accession no. AF355152, and the amino acid sequence of mTLR3 has been reported as GenBank accession no. AAK26117.

Table 4. cDNA Sequence for Murine TLR3

(GenBank Accession No. AF355152; SEQ ID NO:3)

	tagaatatga	tacagggatt	gcacccataa	tctgggctga	atcatgaaag	ggtgttcctc	60
5	ttatctaata	tactcctttg	ggggactttt	gtccctatgg	attcttctgg	tgtcttccac	120
	aaaccaatgc	actgtgagat	acaacgtagc	tgactgcagc	catttgaagc	taacacacat	180
	acctgatgat	cttccctcta	acataacagt	gttgaatctt	actcacaacc	aactcagaag	240
	attaccacct	accaacttta	caagatacag	ccaacttgct	atcttgatg	caggatttaa	300
	ctccatttca	aaactggagc	cagaactgtg	ccaaatactc	cctttgttga	aagtattgaa	360
10	cctgcaacat	aatgagctct	ctcagatttc	tgatcaaacc	tttgtcttct	gcacgaacct	420
	gacagaactc	gatctaata	ctaactcaat	acacaaaatt	aaaagcaacc	ctttcaaaaa	480
	ccagaagaat	ctaatacaat	tagatttgct	tcataatgg	ttatcatcta	caaagttggg	540
	aacgggggtc	caactggaga	acctccaaga	actgctctta	gcaaaaaata	aaatccttgc	600
	gttgcggaag	gaagaacttg	agtttcttgg	caattcttct	ttacgaaagt	tggacttgct	660
15	atcaaatacc	cttaaagagt	tctccccggg	gtgtttccag	acaattggca	agttattcgc	720
	cctcctcttg	aacaacgccc	aactgaaccc	ccacctcaca	gagaagcttt	gctgggaact	780
	ttcaaacaca	agcatccaga	atctctctct	ggctaacaac	cagctgctgg	ccaccagcga	840
	gagcactttc	tctgggctga	agtggacaaa	tctcaccag	ctcgatcttt	cctacaacaa	900
	cctccatgat	gtcggcaacg	gttcccttct	ctatctccca	agcctgaggt	atctgtctct	960
20	ggagtacaac	aatatacagc	gtctgtcccc	tcgctctttt	tatggactct	ccaacctgag	1020
	gtacctgagt	ttgaagcgag	catttactaa	gcaaagtgtt	tcacttgctt	cacatcccaa	1080
	cattgacgat	ttttcctttc	aatgggttaa	atatttggaa	tatctcaaca	tggatgacaa	1140
	taatattcca	agtacccaaa	gcaatacctt	cacgggattg	gtgagtctga	agtacctaa	1200
	tctttccaaa	actttcacaa	gtttgcaaac	tttaacaaat	gaaacatttg	tgtcacttgc	1260
25	tcattctccc	ttgctcactc	tcaacttaac	gaaaaatcac	atctcaaaaa	tagcaaatgg	1320
	tactttctct	tggttaggcc	aactcaggat	acttgatctc	ggccttaatg	aaattgaaca	1380
	aaaactcagc	ggccaggaat	ggagaggctc	gagaaatata	tttgagatct	acctatccta	1440
	taacaaatac	ctccaactgt	ctaccagttc	ccttgcatct	gtccccagcc	ttcaaagact	1500
	gatgctcagg	agggtggccc	ttaaaaatgt	ggatatctcc	ccttcacctt	tccgccctct	1560
30	tcgtaacttg	accattctgg	acttaagcaa	caacaacata	gccaacataa	atgaggactt	1620
	gctggagggt	cttgagaatc	tagaaatcct	ggattttcag	cacaataact	tagccaggct	1680
	ctggaaacgc	gcaaaccccg	gtgggtcccg	taatttctct	aaggggctgt	ctcacctcca	1740
	catcttgaat	ttagagtcca	acggcttaga	tgaaatccca	gtcgggggtt	tcaagaactt	1800
	attcgaacta	aagagcatca	atctaggact	gaataactta	aacaaacttg	aaccattcat	1860
35	ttttgatgac	cagacatctc	taaggctact	gaacctccag	aagaacctca	taacatctgt	1920
	tgagaaggat	gttttcgggc	cgccttttca	aaacctgaac	agtttagata	tgcgcttcaa	1980
	tccgttcgac	tgcacgtgtg	aaagtatttc	ctgggtttgt	aactggatca	accagaccca	2040
	cactaatatc	tttgagctgt	ccactcacta	cctctgtaac	actccacatc	attattatgg	2100
	cttccccctg	aagcttttct	atacatcatc	ctgtaaagac	agcgccccct	ttgaactcct	2160
40	cttcataatc	agcaccagta	tgctcctggg	ttttatactt	gtggtagctg	tcattcacat	2220
	cgagggtctg	aggatctctt	tttactggaa	tgtttcagtg	catcggtatc	ttggtttcaa	2280
	ggaaatagac	acacaggctg	agcagtttga	atatacagcc	tacataattc	atgcccataa	2340
	agacagagac	tgggtctggg	aacattttct	cccaatggaa	gaacaagacc	aatctctcaa	2400
	attttgctta	gaagaaaggg	actttgaagc	aggcgtcctt	ggacttgaag	caattgttaa	2460
45	tagcatcaaa	agaagccgaa	aaatcatttt	cgttatcaca	caccatttat	taaaagacct	2520
	tctgtgcaga	agattcaagg	tacatcacgc	agttcagcaa	gctattgagc	aaaatctgga	2580
	ttcaattata	ctgatttttc	tccagaatat	tccagattat	aaactaaacc	atgcactctg	2640
	tttgcgaaaga	ggaatgttta	aatctcattg	catcttgaac	tggccagttc	agaaagaacg	2700
	gataaatgcc	tttcatcata	aattgcaagt	agcacttgga	tctcggaatt	cagcacatta	2760
50	aactcatttg	aagatttgga	gtcggtaaa	ggatagatcc	aatttataaa	ggtccatcat	2820
	gaatctaagt	tttacttgaa	agttttgtat	atttatttat	atgtatagat	gatgatatta	2880
	catcacaatc	caatctcagt	tttgaaatat	ttcggcttat	ttcattgaca	tctggtttat	2940
	tcaactcaaa	taaacacatg	ggcagttaaa	aacatcctct	attaatagat	taccatttaa	3000
	ttcttgaggt	gtatcacagc	tttaaagggt	tttaaataat	tttatataaa	taagactgag	3060
55	agttttataa	atgtaatttt	ttaaaactcg	agtcttactg	tgtagctcag	aaaggcctgg	3120
	aaattaatat	attagagagt	catgtcttga	acttatttat	ctctgcctcc	ctctgtctcc	3180
	agagtgttgc	ttttaagggc	atgtagcacc	acaccagct	atgtacgtgt	gggattttat	3240


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aatgctcatt ttgagacgt ttatagaata aaagataatt gcttttatgg tataaggcta 3300
cttgaggtaa                                     3310

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Table 5. Amino Acid Sequence for Murine TLR3

5 (GenBank Accession No. AAK26117; SEQ ID NO:4)

	MKGCSSYLMY	SFGGLLSLWI	LLVSSTNQCT	VRYNVADCSH	LKLTHIPDDL	PSNITVLNLT	60
	HNQLRRLPPT	NFTRYSQLAI	LDAGFNSISK	LEPELCQILP	LLKVLNLQHN	ELSQISDQTF	120
	VFCTNLTELD	LMSNSIHKIK	SNPFKNQKNL	IKLDLSHNGI	SSTKLGTGVQ	LENLQELLLA	180
	KNKILALRSE	ELEFLGNSSL	RKLDLSSNPL	KEFSPGCFQT	IGKLFALLLN	NAQLNPHLTE	240
10	KLCWELSNTS	IQNLSLANNQ	LLATSESTFS	GLKWTNLTQL	DLSYNNLHDV	GNGSFSYLP	300
	LRYLSLEYNN	IQRSPRSFY	GLSNLRYLSL	KRAFTKQSVS	LASHPNIDDF	SFQWLKYLEY	360
	LNMDNNIPS	TKSNTFTGLV	SLKYLSTSKT	FTSLQTLTNE	TFVSLAHSPL	LTLNLTKNHI	420
	SKIANGTFSW	LGQLRILDG	LNEIEQKLSG	QEWGRGLRNIF	EIYLSYNKYL	QLSTSSFALV	480
	PSLQRLMLRR	VALKNVDISP	SPFRPLRNLT	ILDLSNNNIA	NINEDLLEGL	ENLEILDFOH	540
15	NNLARLWKRA	NPGGPVNFLK	GLSHLHILNL	ESNGLDEIPV	GVFKNLFELK	SINLGLNNLN	600
	KLEPFIFDDQ	TSLRSLNLQK	NLITSVEKDV	FGPPFQNLNS	LDMRFNPFD	TCESISWVFN	660
	WINQTHTNIF	ELSTHYLCNT	PHYYGFPLK	LFDTSCKDS	APFELLFIIS	TSMLLVFILV	720
	VLLIHIEGWR	ISFYWNVSVH	RILGFKEIDT	QAEQFEYTAY	IIHAHKDRDW	VWEHFSPMEE	780
	QDQSLKFCLE	ERDFEAGVLG	LEAIVNSIKR	SRKIIFVITH	HLLKDPLCRR	FKVHHAVQQA	840
20	IEQNLDIIL	IFLQNPIDYK	LNHALCLRRG	MFKSHCILNW	PVQKERINAF	HHKLQVALGS	900
	RNSAH						905

Example 2. Method of Making IFN- α 4 Reporter Vector

A number of reporter vectors may be used in the practice of the invention.

25 Some of the reporter vectors are commercially available, e.g., the luciferase reporter vectors pNF- κ B-Luc (Stratagene) and pAP1-Luc (Stratagene). These two reporter vectors place the luciferase gene under control of an upstream (5') promoter region derived from genomic DNA for NF- κ B or AP1, respectively. Other reporter vectors can be constructed following standard methods using the desired promoter and a vector
30 containing a suitable reporter, such as luciferase, β -galactosidase (β -gal), chloramphenicol acetyltransferase (CAT), and other reporters known by those skilled in the art. Following are some examples of reporter vectors constructed for use in the present invention.

IFN- α 4 is an immediate-early type 1 IFN. Sequence-specific PCR products for
35 the -620 to +50 promoter region of IFN- α 4 were derived from genomic DNA of human 293 cells and cloned into *Sma*I site of the pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -620 to +50 promoter region of IFN- α 4. The sequence of the -620 to +50 promoter region of IFN- α 4 is provided as SEQ ID NO:11 in Table 6.

Table 6. Nucleotide Sequence of the -620 to +50 Promoter Region of Human IFN- α 4
(SEQ ID NO:11)

	agaaaaattt	taaaaaatta	ttcattcata	tttttaggag	ttttgaatga	ttggatatgt	60
	aattatattc	atattattaa	tgtgtatcta	tatagatttt	tattttgcat	atgtactttg	120
5	atacaaaatt	tacatgaaca	aattacacta	aaagttattc	cacaaatata	cttatcaaat	180
	taagttaa	gtcaatagct	tttaaactta	aatttttagt	taacttttct	gtcattcttt	240
	actttgaata	aaaagagcaa	actttgtagt	ttttatctgt	gaagtagagg	tatacgtaat	300
	atacataaat	agatatgcca	aatctgtgtt	attaaaattt	catgaagatt	tcaattagaa	360
	aaaaatacca	taaaaggctt	tgagtgcagg	tgaaaaatag	gcaatgatga	aaaaaatga	420
10	aaaacttttt	aaacacatgt	agagagtgcg	taaagaaagc	aaaaacagag	atagaaagta	480
	caactaggga	atttagaaaa	tggaaattag	tatgttcact	atttaagacc	tatgcacaga	540
	gcaaagtctt	cagaaaacct	agaggccgaa	gttcaagggt	atccatctca	agtagcctag	600
	caatatttgc	aacatcccaa	tggccctgtc	cttttcttta	ctgatggccg	tgctggtgct	660
	cagctacaaa						670
15							

Example 3. Method of Making IFN- α 1 Reporter Vector

IFN- α 1 is a late type 1 IFN. Sequence-specific PCR products for the -140 to +9 promoter region of IFN- α 1 were derived from genomic DNA of human 293 cells and cloned into *Sma*I site of the pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -140 to +9 promoter region of IFN- α 1.

Example 4. Method of Making IFN- β Reporter Vector

IFN- β is an immediate-early type 1 IFN. The -280 to +20 promoter region of IFN- β was derived from the pUC β 26 vector (Algarté M et al. (1999) *J Virol* 73(4):2694-702) by restriction at *Eco*RI and *Taq*I sites. The 300 bp restriction fragment was filled in by Klenow enzyme and cloned into *Nhe*I-digested and filled in pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -280 to +20 promoter region of IFN- β . The sequence of the -280 to +20 promoter region of IFN- β is provided as SEQ ID NO:12 in Table 7.

Table 7. Nucleotide Sequence of the -280 to +20 Promoter Region of Human IFN- β
(SEQ ID NO:12)

	ttctcaggtc	gtttgctttc	ctttgctttc	tcccaagtct	tgttttacaa	tttgcttttag	60
35	tcattcactg	aaactttaaa	aaacattaga	aaacctcaca	gtttgtaaat	ctttttccct	120
	attatatata	tcataagata	ggagcttaaa	taaagagttt	tagaaactac	taaaatgtaa	180
	atgacatagg	aaaactgaaa	gggagaagtg	aaagtgggaa	attcctctga	atagagagag	240
	gaccatctca	tataaatagg	ccatacccac	ggagaaagga	cattctaact	gcaacctttc	300

Example 5. Method of Making RANTES Reporter Vector

Transcription of the chemokine RANTES is believed to be regulated at least in part by IRF3 and by NF- κ B. Lin R et al. (1999) *J Mol Cell Biol* 19(2):959-66; Genin P et al. (2000) *J Immunol* 164:5352-61. A 483 bp sequence-specific PCR product

5 including the -397 to +5 promoter region of RANTES was derived from genomic DNA of human 293 cells, restricted with PstI and cloned into pCAT-Basic Vector (Promega) using HindIII (filled in with Klenow) and PstI sites (filled in). The -397 to +5 promoter region of RANTES was then isolated from the resulting

RANTES/chloramphenicol acetyltransferase (CAT) reporter plasmid by restriction with

10 BglII and SalI, filled in with Klenow enzyme, and cloned into the NheI site (filled in with Klenow) of the pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -397 to +5 promoter region of RANTES. Comparison of the insert sequence -397 to +5 of Genin P et al. (2000) *J Immunol* 164:5352-61 and GenBank accession no. AB023652 (SEQ ID NO:13)

15 revealed two point deletions (at positions 105 and 273 of SEQ ID NO:13) which do not create new restriction sites. The sequence of the -397 to +5 promoter region of RANTES is provided as SEQ ID NO:14 in Table 8.

Table 8. Nucleotide Sequence of the -397 to +5 Promoter Region of Human RANTES (SEQ ID NO:14)

20	gatctgtaat	gaataagcag	gaactttgaa	gactcagtga	ctcagtgagt	aataaagact	60
	cagtgacttc	tgatcctgtc	ctaactgcca	ctccttggtg	tcccaagaaa	gcggcttcct	120
	gctctctgag	gaggaccctt	tccttggaag	gtaaaactaa	ggatgtcagc	agagaaattt	180
	ttccaccatt	ggtgcttggt	caaagaggaa	actgatgagc	tcactctaga	tgagagagca	240
25	gtgagggaga	gacagagact	cgaatttccg	gagctatttc	agttttcttt	tccgttttgt	300
	gcaatttcac	ttatgatacc	ggccaatgct	tggttgctat	tttggaact	ccccttaggg	360
	gatgcccctc	aactggcctt	ataaagggcc	agcctgagct	g		401

Table 9. Nucleotide Sequence of GenBank Accession No. AB023652 (SEQ ID NO:13)

30	agaaggcctt	acagtgagat	gggatcccag	tatttattga	gtttcctcat	tcataaaatg	60
	gggataataa	tagtaaataa	gttgacacgc	gctaagacag	tggaatagtg	gctggcacag	120
	ataagccctc	ggtaaatagt	agccaataat	gatagagtat	gctgtaagat	atctttctct	180
	ccctctgctt	ctcaacaagt	ctctaataca	ttattccact	ttataaacia	ggaaatagaa	240
	ctcaaagaca	ttaagcactt	ttcccaaagg	tcgcttagca	agtaaatggg	agagacccta	300
35	tgaccaggat	gaaagcaaga	aattcccaca	agaggactca	ttccaactca	tatcttgtga	360
	aaagggtccc	aatgcccagc	tcagatcaac	tgccctcaatt	tacagtgtga	gtgtgctcac	420
	ctcctttggg	gactgtatat	ccagaggacc	ctcctcaata	aaacacttta	taaataacat	480
	ccttccatgg	atgagggaaa	ggaggtaaga	tctgtaatat	ataagcagga	actttgaaga	540
	ctcagtgact	cagtgagtaa	taaagactca	gtgacttctg	atcctgtcct	aactgccact	600
40	ccttggtgtc	cccaagaaag	cggcttcctg	ctctctgagg	aggaccctt	ccctggaagg	660
	taaaactaag	gatgtcagca	gagaaatttt	tccaccattg	gtgcttggtc	aaagaggaaa	720

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ctgatgagct cactctagat gagagagcag tgagggagag acagagactc gaatttcg 780
aggctatttc agttttcttt tccgttttgt gcaatttcac ttatgatacc ggccaatgct 840
tggttgctat tttggaaact ccccttaggg gatgccctc aactggcctc ataaagggcc 900
agcctgagct gcagaggatt cctgcagagg atcaagacag cacgtggacc tcgcacagcc 960
5 tctcccacag gtaccatgaa ggtctccgcg gcagccctcg ctgtcatcct cattgctact 1020
gccctctgcg c 1031

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Example 6. Method of Making Human IL-12 p40 Reporter Vectors

Reporter constructs have been made using truncated (-250 to +30) and full
length (-860 to +30) promoter regions derived from human IL-12 p40 genomic DNA.
In one reporter construct the truncated IL-12 p40 promoter was cloned as a KpnI-XhoI
insert into p β gal-Basic (Promega). The resulting expression vector includes a β gal
gene under control of an upstream (5') -250 to +30 promoter region of human IL-12
p40. In a second reporter construct the full length IL-12 p40 promoter was cloned as a
15 KpnI-XhoI insert into p β gal-Basic (Promega). The resulting expression vector includes
a β gal gene under control of an upstream (5') -860 to +30 promoter region of human
IL-12 p40. In a third reporter construct the truncated -250 to +30 promoter region of
human IL-12 p40 was cloned into the pGL3-Basic Vector (Promega). The resulting
expression vector includes a luciferase gene under control of an upstream (5') -250 to
20 +30 promoter region of human IL-12 p40. In a fourth reporter construct the full length
IL-12 p40 promoter of human IL-12 p40 was cloned into the pGL3-Basic Vector
(Promega). The resulting expression vector includes a luciferase gene under control of
an upstream (5') -860 to +30 promoter region of human IL-12 p40.

25 Example 7. Method of Making Human IL-6 Reporter Vectors

Reporter constructs are made using the -235 to +7 promoter region derived from
human IL-6 genomic DNA. In one reporter construct the IL-6 promoter region is
cloned as a KpnI-XhoI insert into pGL3-Basic Vector (Promega). The resulting
expression vector includes a luciferase gene under control of an upstream (5') -235 to
30 +7 promoter region derived from human IL-6 genomic DNA.

Example 8. Method of Making Human IL-8 Reporter Vectors

Reporter constructs have been made using a -546 to +44 and a truncated -133 to
+44 promoter region derived from human IL-8 genomic DNA. Mukaida N et al.
35 (1989) *J Immunol* 143:1366-71. In each reporter construct the IL-8 promoter region

was cloned as a KpnI-XhoI insert into pGL3-Basic Vector (Promega). One of the resulting expression vectors includes a luciferase gene under control of an upstream (5') -546 to +44 promoter region derived from human IL-8 genomic DNA. Another of the resulting expression vectors includes a luciferase gene under control of an upstream (5')
5 -133 to +44 promoter region derived from human IL-8 genomic DNA.

Example 9. Sequence Comparison of Human TLR3 and Human TLR9

Human TLR3 and TLR9 are homologous proteins with several structural commonalities. Both appear to be transmembrane proteins with an extracellular
10 domain and an intracellular domain. Common characteristics include a signal sequence and transmembranal domain. Similarities common to most TLRs include a cysteine rich domain and a TIR domain. Most TLRs have leucine rich repeats (LRR) in their extracellular domain. TLR3, TLR7, TLR8, and TLR9 appear to have similar structures. The regularity of the leucine repeats are shown below for TLR3 and TLR9.
15 These four TLRs can be broken into two extracellular subdomains, domain 1 and 2, by virtue of a separation by an unstructured hinge region. TLR7, TLR8, and TLR9 have 14 LRR in domain 1 and 12 LRR in domain 2. TLR9 is a known nucleic acid binder, interacting with CpG-DNA. It has been suspected that TLR7 and TLR8 most likely also interact with nucleic acids. TLR3 has a similar 11 LRR in domain 1 and has 12
20 LRR in domain 2, lacking the initial 3 repeats common to TLR7, TLR8, and TLR9. Based on structural consideration it is hypothesized that TLR3 interacts with nucleic acids or similar structures.

The structure of TLR3 differs from TLR7, TLR8, and TLR9 in an interesting character. Referring to Table 13, within the TIR domain it has been shown that a
25 proline (shown in bold) is required for MyD88 interaction. MyD88 is required for TLR9 to transduce signal for the activation of NF-κB. Both TLR7 and TLR8 also have this proline. TLR3 however has an alanine at this position (also shown in bold). It is believed by the applicant that this difference may disallow MyD88 interaction with TLR3 and thus result in an altered signal transduction pattern compared to, e.g., TLR9.

30

Table 10. Sequence Alignment of hTLR9 (SEQ ID NO:6) and hTLR3 (SEQ ID NO:2)

SIGNAL SEQUENCE

	hTLR9	MGFCRSALHPLSLLVQAIMLAMTLALGTLPAFLPCELQPHGLVNCNW	47
	hTLR3	MRQTLPCIYFWGGLLPFGMLCASSTTKCTVSHEVADC	37
DOMAIN 1 LEUCINE RICH REPEATS			
5	hTLR9	LFLKSVPHFMSMAAPRGNVTSLSLSSN	73
	hTLR9	RIHHLHDSDFAHLP SLRHLNLKWN	97
10	hTLR9	CPPVGLSPMHFPCHMTIEPSTFLAVPTLEELNLSYN	133
	hTLR9	NIMTVPALPKSLISLSLSHT	153
15	hTLR3	SHLKL TQVPDDLPTNITVLNLTHN	61
	hTLR9	NILMLDSASLAGLHALRFLFMDGN	177
	hTLR3	QLRRLPAA NFTRYSQLTSLDVGFN	85
20	hTLR9	CYYKNPCRQALEVAPGALLGLGNLTHLSLKYN	209
	hTLR3	TISKLEPELCQKLPMLKVLNLQHN	109
	hTLR9	NLTVVPRNLPSSLEYLLLSYN	230
	hTLR3	ELSQLSDKTFAFCTNLTELHLSN	133
25	hTLR9	RIVKLAPEDLANLTALRVLDVGGN	254
	hTLR3	SIQKIKNNPFVKQKNLITLDLSHN	157
	hTLR9	<u>CRRCDHAPNPCMECPRHFPQLHPDTFSHL SRLEGLVLKDS</u>	294
30	hTLR3	GLSSTKLGTQVQLENLQELLSNN	181
	hTLR9	SLSWLNASWFRGLGNLRVLDLSEN	318
	hTLR3	KIQALKSEELDI FANSSLKKLELSSN	207
35	hTLR9	FLYKCITKTKAFQGLTQLRKLNLSEFN	344
	hTLR3	QIKEFSPGCFHAIGRLFGLFLNNV	231
	hTLR9	YQKRVSFAHLSLAPSEFGLVALKELDMHGI	374
	hTLR3	QLGPSLTEKLCLELANTSIRNLSLSNS	258
40	hTLR9	FFRSLDETTLRPLARLPMLQTLRLQMN	401
	hTLR3	QLSTTSNTTFLGLKWTNLTMLDLSYN	284
	hTLR9	FINQAQLGIFRAFPGLRYVDLSDN	425
45	hTLR3	NLNVVGND SFAWLPQLEYFFLEYN	308
HINGE REGION			
	hTLR9	RISGASELTATMGEADGGEKVWLQPGDLAPAPV	458
	hTLR3	NIQH LFSHSLHGLFNVRYNLKR SFTKQSI SLA	341
50	DOMAIN 2 LEUCINE RICH REPEATS		
	hTLR9	DTPSSEDFRPNCSTLNFTLDLSRN	482
	hTLR3	SLPKIDDFS FQWLKCLEHLNMEDN	365
55	hTLR9	NLVTVPQEMFAQLSHLQCLRLSHN	506
	hTLR3	DIPGIKSNMFTGLINLKYLSLSNS	389

	hTLR9	CISQAVNGSQFLPLTGQVLDLSHN	531
	hTLR3	FTSLRTLNETFVSLAHSPLHILNLTKN	417
5	hTLR9	KLDLYHEHSFTELPRLEALDLSYN	555
	hTLR3	KISKIESDAFSWLGHLEVLDLGLN	441
	hTLR9	SQPFQMGGVGHNFVVAHLRTLRLSLAHN	585
10	hTLR3	EIGQELTGQEWGLENIFEIYLSYN	466
	hTLR9	NIHSQVSQQLCSTSLRALDFSGN	608
	hTLR3	KYLQLTRNSFALVPSLQRLMLRRV	490
15	hTLR9	ALGHMWAEGDLYLHFFQGLSGLIWLDLSQN	638
	hTLR3	ALKNVDSSPSPFQPLRNLTLILDLSNN	516
	hTLR9	RLHTLLPQTLRNLPKSLQVLRRLDN	663
	hTLR3	NIANINDDMLEGLEKLEILDLOHN	540
20	hTLR9	YLAFKWWSLHFLPKLEVLDLAGN	687
	hTLR3	NLARLWKHANPGGPIYFLKGLSHLHILNLESN	572
	hTLR9	QLKALTNGSLPAGTRLRRLDVSCN	711
25	hTLR3	GFDEIPVEVFKDLFELKIIDLGLN	596
	hTLR9	SISFVAPGFFSKAKELRELNLSAN	735
	hTLR3	NLNTLPASVFNNQVSLKSLNLQKN	620
30	hTLR9	ALKTVDHSWFGPLASALQILDVSAN	760
	hTLR3	LITSVEKKVFGPAFRNLTELDMRFN	645
CYSTEINE RICH DOMAIN			
35	hTLR9	PLHCACG**AAFMDFLLEVQAAVPGLPSRVKCGSPGQLQGLSIFAQD	805
	hTLR3	PFDCCTCESIAWFVNWINETHTNIPELSSHYLCNTPPHYHGFPVRLFD	692
	hTLR9	LRLCLDEALSWDCFA	820
	hTLR3	TSSCKDSAPFELFFM	707
TRANSMEMBRANAL DOMAIN			
40	hTLR9	LSLLAVALGLGVPMHLHL	838
	hTLR3	INTSILLIFIFIVLLIHF	725
TIR DOMAIN			
45	hTLR9	CGWDLWYCFHLCLAWLPWRGRQSGRDEDALPYDAFVVFDKTQSAVAD	885
	hTLR3	EGWRISFYWNVSVHRVLGFKEIDRQTEQFE*YAAYIIHAYK***DKD	768
	hTLR9	WVYNELRGQLEECRGRWALRLCLEERDWLPGKTLFENLWASVYGSRK	932
	hTLR3	WVW***EHFSSMEKEDQSLKFCLEERDFEAGVFELEAIVNSIKRSRK	812
50	hTLR9	TLFVLAHTD*RVSGLLRASFLLAQQRLLEDKRDVVVLVILSPDGRRS	978
	hTLR3	IIFVITHHLLKDPLCKRFKVHHAQQAEQNLDSEILVFLEEIPDYK	859
	hTLR9	***RYVRLRQRLCRQSVLLWPHQPSGQRSFWAQLGMALTRDNHHFYN	1022
55	hTLR3	LNHALCLRRGMFKSHCILNWPVQKERIGAFRHKLQVALGSKNSVH	904
	hTLR9	RNFCQGPTAE	1032

Example 10. Reconstitution of TLR9 Signaling in 293 Fibroblasts

Methods for cloning murine and human TLR9 have been described in pending U.S. Patent Application No. 09/954,987 and corresponding published PCT application
 5 PCT/US01/29229, both filed September 17, 2001, the contents of which are incorporated by reference. Human TLR9 cDNA and murine TLR9 cDNA in pT-Adv vector (from Clontech) were individually cloned into the expression vector pcDNA3.1(-) from Invitrogen using the EcoRI site. Utilizing a “gain of function” assay it was possible to reconstitute human TLR9 (hTLR9) and murine TLR9 (mTLR9)
 10 signaling in CpG-DNA non-responsive human 293 fibroblasts (ATCC, CRL-1573). The expression vectors mentioned above were transfected into 293 fibroblast cells using the calcium phosphate method.

Table 11. cDNA Sequence for Human TLR9

15 (GenBank Accession No. AF245704; SEQ ID NO:5)

	aggctggtat	aaaaatctta	cttccctctat	tctctgagcc	gctgctgccc	ctgtgggaag	60
	ggacctcgag	tgtgaagcat	ccttccctgt	agctgctgtc	cagtctgccc	gccagaccct	120
	ctggagaagc	ccctgcccc	cagcatgggt	ttctgccgca	gcgcctgca	ccgctgtct	180
	ctcctggtgc	aggccatcat	gctggccatg	accctggccc	tgggtacctt	gcctgccttc	240
20	ctaccctgtg	agctccagcc	ccacggcctg	gtgaactgca	actggctgtt	cctgaagtct	300
	gtgccccact	tctccatggc	agcaccctgt	ggcaatgtca	ccagcctttc	cttgtcctcc	360
	aaccgcatcc	accacctcca	tgattctgac	tttgcccacc	tgcccagcct	gcggcatctc	420
	aacctcaagt	ggaactgccc	gccggttgge	ctcagcccca	tgcacttccc	ctgccacatg	480
	accatcgagc	ccagcacctt	cttggctgtg	cccaccctgg	aagagctaaa	cctgagctac	540
25	aacaacatca	tgactgtgcc	tgcgctgccc	aaatccctca	tatccctgtc	cctcagccat	600
	accaacatcc	tgatgctaga	ctctgccagc	ctcgccggcc	tgcatgccct	gcgcttccta	660
	ttcatggacg	gcaactgtta	ttacaagaac	ccctgcaggc	aggcactgga	ggtggccccg	720
	ggtgccctcc	ttggcctggg	caacctcacc	cacctgtcac	tcaagtacaa	caacctcact	780
	gtggtgcccc	gcaacctgcc	ttccagcctg	gagtatctgc	tgttgctccta	caaccgcata	840
30	gtcaaaactgg	cgcttgagga	cctggccaat	ctgaccgccc	tgcgtgtgct	cgatgtgggc	900
	ggaaattgcc	gccgctgcga	ccacgctccc	aacccttgca	tggagtggcc	tcgtcacttc	960
	ccccagctac	atcccagatac	cttcagccac	ctgagccgtc	ttgaaggcct	ggtgttgaag	1020
	gacagtcttc	tctcctggct	gaatgccagt	tgggtccgtg	ggctgggaaa	cctccgagtg	1080
	ctggacctga	gtgagaactt	cctctacaaa	tgcataccta	aaaccaaggc	cttccagggc	1140
35	ctaacacagc	tgcgcaagct	taacctgtcc	ttcaattacc	aaaagagggt	gtcctttgcc	1200
	cacctgtctc	tggccccttc	cttcggggagc	ctggctcgccc	tgaaggagct	ggacatgcac	1260
	ggcatcttct	tccgctcact	cgatgagacc	acgctccggc	cactggcccg	cctgcccata	1320
	ctccagactc	tgcgctcgca	gatgaacttc	atcaaccagg	cccagctcgg	catcttcagg	1380
	gccttccctg	gcctgcgcta	cgtggacctg	tcggacaacc	gcatcagcgg	agcttcggag	1440
40	ctgacagcca	ccatggggga	ggcagatgga	ggggagaagg	tctggctgca	gcctggggac	1500
	cttgctccgg	ccccagtgga	cactcccagc	tctgaagact	tcaggcccaa	ctgcagcacc	1560
	ctcaacttca	ccttggatct	gtcacggaac	aacctggtga	ccgtgcagcc	ggagatgttt	1620
	gcccagctct	cgcacctgca	gtgcctgcgc	ctgagccaca	actgcatctc	gcaggcagtc	1680
	aatggctccc	agttcctgcc	gctgaccggg	ctgcaggtgc	tagacctgtc	ccgcaataag	1740
45	ctggacctct	accacgagca	ctcattcacg	gagctaccgc	gactggaggc	cctggacctc	1800
	agctacaaca	gccagccctt	tggcatgcag	ggcggtgggc	acaacttcag	cttcgtggct	1860
	cacctgcgca	ccctgcgcca	cctcagcctg	gcccacaaca	acatccacag	ccaagtgtcc	1920

	cagcagctct	gcagtacgtc	gctgcggggc	ctggacttca	gcggcaatgc	actgggccaat	1980
	atgtggggcg	aggagagacct	ctatctgcac	ttcttccaag	gcctgagcgg	tttgatctgg	2040
	ctggacttgt	cccagaaccg	cctgcacacc	ctcctgcccc	aaaccctgcg	caacctcccc	2100
	aagagcctac	agggtgctgcg	tctccgtgac	aattacctgg	ccttctttaa	gtggtggagc	2160
5	ctccacttcc	tgcccaaact	ggaagtcctc	gacctggcag	gaaaccggct	gaaggccctg	2220
	accaatggca	gcctgcctgc	tggcaccg	ctccggaggc	tggatgtcag	ctgcaacagc	2280
	atcagcttcg	tggcccccgg	cttcttttcc	aaggccaagg	agctgcgaga	gctcaacctt	2340
	agcgccaacg	ccctcaagac	agtggaccac	tcctggtttg	ggcccctggc	gagtgccttg	2400
	caaatactag	atgtaagcgc	caaccctctg	cactgcgcct	gtggggcggc	ctttatggac	2460
10	ttcctgctgg	agggtgcaggc	tgccgtgccc	ggctgcccc	gccgggtgaa	gtgtggcagt	2520
	ccggggccagc	tccagggcct	cagcatcttt	gcacaggacc	tgcgcctctg	cctggatgag	2580
	gccctctcct	gggactgttt	cgcctctctg	ctgctggctg	tggctctggg	cctgggtgtg	2640
	cccatgctgc	atcacctctg	tggctgggac	ctctgggtact	gcttccacct	gtgcctggcc	2700
	tggcttccct	ggcggggggcg	gcaaagtggg	cgagatgagg	atgccctgcc	ctacgatgcc	2760
15	ttcgtggtct	tcgacaaaac	gcagagcgca	gtggcagact	gggtgtacaa	cgagcttcgg	2820
	gggcagctgg	aggagtgcg	tgggcgctgg	gcactccgcc	tgtgcctgga	ggaacgcgac	2880
	tggctgcctg	gcaaaaccct	ctttgagaac	ctgtgggcct	cggctctatg	cagccgcaag	2940
	acgctgtttg	tgetggccca	cacggaccgg	gtcagtggtc	tcttgccgcg	cagcttcctg	3000
	ctggcccagc	agcgccctgct	ggaggaccgc	aaggacgtcg	tgggtgctggt	gatcctgagc	3060
20	cctgacggcc	gccgctcccg	ctacgtgcgg	ctgcgccagc	gcctctgccg	ccagagtgtc	3120
	ctcctctggc	cccaccagcc	cagtggtcag	cgcagcttct	gggccagct	gggcatggcc	3180
	ctgaccaggg	acaaccacca	cttctataac	cggaaacttct	gccagggacc	cacggccgaa	3240
	tagccgtgag	ccggaatcct	gcacgggtgcc	acctccacac	tcacctcacc	tctgcctgcc	3300
	tggctctgacc	ctcccctgct	cgcctccctc	acccacacac	tgacacagag	ca	3352
25							

Table 12. Amino Acid Sequence for Human TLR9

(GenBank Accession No. AAF78037; SEQ ID NO:6)

	MGFCRSALHP	LSLLVQAIML	AMTLALGTLP	AFLPCELQPH	GLVNCNWLFL	KSVPHFSMAA	60
	PRGNVTSLSL	SSNRIHHLHD	SFAHLPSLR	HLNLKWNCP	VGLSPMHFPC	HMTIEPSTFL	120
30	AVPTLEELNL	SYNNIMTVPA	LPKSLISLSL	SHTNILMLDS	ASLAGLHALR	FLFMDGNCY	180
	KNPCRQALEV	APGALLGLGN	LTHLSLKYN	LTVVPRNLPS	SLEYLLLSYN	RIVKLAPEDL	240
	ANLTALRVLD	VGGNCRRCDH	APNPCMECPR	HFPQLHPDTF	SHLSRLEGLV	LKDSSLSWLN	300
	ASWFRGLGNL	RVLDSLSEFL	YKCITKTKAF	QGLTQLRKLN	LSFNYQKRVS	FAHLSLAPSF	360
	GSLVALKELD	MHGIFFRSLD	ETTLRPLARL	PMLQTLRLQM	NFINQAQLGI	FRAFPGLRYV	420
35	DLSDNRISGA	SELTATMGEA	DGGEKVWLQP	GDLAPAPVDT	PSEDFRPNC	STLNFTLDLS	480
	RNNLVTVQPE	MFAQLSHLQC	LRLSHNCISQ	AVNGSQFLPL	TGLQVLDLSR	NKLDLYHEHS	540
	FTELPRLEAL	DLSYNSQPF	MQGVGHNF	VAHLRTLRLH	SLAHNNIHSQ	VSQQLCSTSL	600
	RALDFSGNAL	GHMWAEGDLY	LHFFQGLSGL	IWLDLSQNRL	HTLLPQTLRN	LPKSLQVLRL	660
	RDNYLAFFKW	WSLHFLPKLE	VLDLAGNRLK	ALTNGSLPAG	TRLRLDVSC	NSISFVAPGF	720
40	FSKAKELREL	NLSANALKTV	DHSWFGPLAS	ALQILDVSAN	PLHCACGAAF	MDFLLEVQAA	780
	VPGLPSRVKC	GSPGQLQGLS	IFAQDLRLCL	DEALSWDCFA	LSLLAVALGL	GVPMLHHLCG	840
	WDLWYCFHLC	LAWLPWRGRQ	SGRDEDALPY	DAFVVFDKTQ	SAVADWVYNE	LRGQLEECRG	900
	RWALRLCLEE	RDWLPKKTFL	ENLWASVYGS	RKTLFVLAHT	DRVSGLLRAS	FLLAQQRLLE	960
	DRKDVVVLVI	LSPDGRRSRY	VRLRQRLCRQ	SVLLWPHQPS	GQRSFWAQLG	MALTRDNHFF	1020
45	YNRNFCQGPT	AE					1032

Table 13. cDNA Sequence for Murine TLR9

(GenBank Accession No. AF348140; SEQ ID NO:7)

	tgtcagaggg	agcctcgagg	gaatcctcca	tctcccaaca	tggttctccg	tcgaaggact	60
50	ctgcacccct	tgtccctcct	ggtacaggct	gcagtgtctg	ctgagactct	ggccctgggt	120
	accctgcctg	ccttctctac	ctgtgagctg	aagcctcatg	gcctggtgga	ctgcaattgg	180
	ctgttctctga	agtctgtacc	ccgtttctct	gcggcagcat	cctgctccaa	catcaccgcg	240
	ctctccttga	tctccaaccg	tatccaccac	ctgcacaact	ccgacttcgt	ccacctgtcc	300
	aacctgcggc	agctgaacct	caagtgggaa	tgtccaccca	ctggccttag	ccccctgcac	360

	ttctcttgcc	acatgaccat	tgagcccaga	accttcctgg	ctatgcgtac	actggaggag	420
	ctgaacctga	gctataatgg	tatcaccact	gtgccccgac	tgcccagctc	cctgggtgaat	480
	ctgagcctga	gccacaccaa	catcctgggt	ctagatgcta	acagcctcgc	cggcctatac	540
	agcctgcgcg	ttctcttcat	ggacgggaac	tgctactaca	agaacccctg	cacaggagcg	600
5	gtgaagggtga	ccccaggcgc	cctcctgggc	ctgagcaatc	tcacccatct	gtctctgaag	660
	tataacaacc	tcacaaaggt	gccccgcaa	ctgcccccca	gcctggagta	cctcctgggtg	720
	tcctataacc	tcattgtcaa	gctggggcct	gaagacctgg	ccaatctgac	ctcccttcga	780
	gtacttgatg	tgggtgggaa	ttgccgtcgc	tgcgaccatg	cccccaatcc	ctgtatagaa	840
	tgtggccaaa	agtccttcca	cctgcaccct	gagaccttcc	atcacctgag	ccatctggaa	900
10	ggcctgggtgc	tgaaggacag	ctctctccat	acactgaact	cttcctgggt	ccaaggctctg	960
	gtcaacctct	cgggtgctgga	cctaagcgag	aactttctct	atgaaagcat	caaccacacc	1020
	aatgcctttc	agaacctaac	ccgcctgcgc	aagctcaacc	tgtccttcaa	ttaccgcaag	1080
	aaggatcct	ttgcccgcct	ccacctggca	agttccttca	agaacctggg	gtcactgcag	1140
	gagctgaaca	tgaacggcat	cttcttccgc	tcgctcaaca	agtacacgct	cagatggctg	1200
15	gccgatctgc	ccaaactcca	cactctgcat	cttcaaataga	acttcatcaa	ccaggcacag	1260
	ctcagcatct	ttggtacctt	ccgagccctt	cgctttgtgg	acttgtcaga	caatcgcate	1320
	agtgggcctt	caacgctgtc	agaagccacc	cctgaagagg	cagatgatgc	agagcaggag	1380
	gagctgttgt	ctgcggatcc	tcacccagct	ccactgagca	cccctgcttc	taagaacttc	1440
	atggacaggt	gtaagaactt	caagttcacc	atggacctgt	ctcggaacaa	cctgggtgact	1500
20	atcaagccag	agatgtttgt	caatctctca	cgcctccagt	gtcttagcct	gagccacaac	1560
	tccattgcac	aggctgtcaa	tggctctcag	ttcctgccgc	tgactaatct	gcagggtgctg	1620
	gacctgtccc	ataacaaact	ggacttgtac	cactggaaat	cgttcagtga	gctaccacag	1680
	ttgcaggccc	tggacctgag	ctacaacagc	cagcccttta	gcatgaaggg	tataggccac	1740
	aatttcagtt	ttgtggccca	tctgtccatg	ctacacagcc	ttagcctggc	acacaatgac	1800
25	attcataccc	gtgtgtcctc	acatctcaac	agcaactcag	tgaggtttct	tgacttcagc	1860
	ggcaacggta	tgggccgcat	gtgggatgag	gggggccttt	atctccattt	cttccaagge	1920
	ctgagtggcc	tgctgaagct	ggacctgtct	caaaataacc	tgcataatct	ccggccccag	1980
	aaccttgaca	acctccccaa	gagcctgaag	ctgctgagcc	tccgagacaa	ctacctatct	2040
	ttcttttaact	ggaccagtct	gtccttcctg	cccaacctgg	aagtcctaga	cctgggcaggc	2100
30	aaccagctaa	aggccctgac	caatggcacc	ctgcctaata	gcacctcct	ccagaaactg	2160
	gatgtcagca	gcaacagtat	cgtctctgtg	gtcccagcct	tcttcgctct	ggcggtcgag	2220
	ctgaaagagg	tcaacctcag	ccacaacatt	ctcaagacgg	tggatcgctc	ctgggtttggg	2280
	cccattgtga	tgaacctgac	agttctagac	gtgagaagca	accctctgca	ctgtgcctgt	2340
	ggggcagcct	tcgtagactt	actgttggag	gtgcagacca	aggtgcctgg	cctggctaata	2400
35	ggtgtgaagt	gtggcagccc	cggccagctg	cagggccgta	gcatcttcgc	acaggacctg	2460
	cggctgtgcc	tggatgaggt	cctctcttgg	gactgctttg	gcctttcact	cttggctgtg	2520
	gccgtgggca	tgggtgggtgc	tatactgcac	catctctgcg	gctgggacgt	ctgggtactgt	2580
	tttcatctgt	gcctggcatg	gctacctttg	ctggccccga	gccgacgcag	cgcccaagct	2640
	ctccccatg	atgccttcgt	ggtgttcgat	aaggcacaga	gcgcagttgc	ggactgggtg	2700
40	tataacgagc	tgccgggtgcg	gctggaggag	cggcgcggtc	gccgagccct	acgcttgtgt	2760
	ctggaggacc	gagattggct	gcctggccag	acgctcttcg	agaacctctg	ggcttccate	2820
	tatgggagcc	gcaagactct	atthgtgctg	gcccacacgg	accgcgtcag	tggcctcctg	2880
	cgcaccagct	tcctgctggc	tcagcagcgc	ctgttggaag	accgcaagga	cgtgggtggg	2940
	ttgggtgatcc	tgcgtccgga	tgccaccgcg	tcccgtatg	tgcgactgcg	ccagcgtctc	3000
45	tgccgccaga	gtgtgctctt	ctggccccag	cagcccaacg	ggcagggggg	cttctggggc	3060
	cagctgagta	cagccctgac	tagggacaac	cgccacttct	ataaccagaa	cttctgccgg	3120
	ggacctacag	cagaatagct	cagagcaaca	gctggaaaca	gctgcatctt	catgcctggg	3180
	tcccagagttg	ctctgcctgc					3200

50 Table 14. Amino Acid Sequence for Murine TLR9

(GenBank Accession No. AAK29625; SEQ ID NO:8)

	MVLRRRTLHP	LSLLVQAAVL	AETLALGTLP	AFLPCELKPH	GLVDCNWLFL	KSVPRFSAAA	60
	SCSNITRLSL	ISNRIHHLHN	SDFVHLSNLR	QLNLKWNCP	TGLSPLHFSC	HMTIEPRTFL	120
	AMRTLEELNL	SYNGITTVPR	LPSSLVNLSL	SHTNIVLDA	NSLAGLYSLR	VLFMDGNCYY	180
55	KNPCTGAVKV	TPGALLGLSN	LTHLSLKYN	LTKVPRQLPP	SLEYLLVSYN	LIVKLGPEDL	240
	ANLTSRLVLD	VGGNCRCDH	APNPCIECGQ	KSLHLHPETF	HHLSHLEGLV	LKDSSLHTLN	300
	SSWFQGLVNL	SVLDLSENFL	YESINHNTAF	QNLTRLRKL	LSFNRYRKKVS	FARLHLASSF	360

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KNLVSLQELN MNGIFFRSLN KYTLRWLADL PKLHTLHLQM NFINQAQLSI FGTFRALRFV 420
DLSDNRISGP STLSEATPEE ADDAEQEELL SADPHPAPLS TPASKNFMDR CKNFKFTMDL 480
SRNNLVTIKP EMFVNLSRLQ CLSLSHNSIA QAVNGSQFLP LTNLQVLDLS HNKLDLYHWK 540
SFSELPQLQA LDLSYNSQPF SMKGIGHNFS FVAHLSMLHS LSLAHNDIHT RVSSHLNSNS 600
5 VRFLDFSGNG MGRMWDEGGL YLHFFQGLSG LLKLDLSQNN LHILRPQNLD NLPKSLKLLS 660
LRDNYLSFFN WTSLSFLPNL EVLDLAGNQL KALTNGTLPN GTLLQKLDVS SNSIVSVVPA 720
FFALAVELKE VNLSHNILKT VDRSWFGPIV MNLTVLDVRS NPLHCACGAA FVDLLLEVQT 780
KVPGLANGVK CGSPGQLQGR SIFAQDLRLC LDEVLSWDCF GLSLLAVAVG MVVPILHHLC 840
GWDVWYCFHL CLAWLPLLAR SRRSAQALPY DAFVVFDKAQ SAVADWVYNE LRVRLERRG 900
10 RRALRLCLED RDWLPQTTLF ENLWASIYGS RKTFLVLAHT DRVSGLLRTS FLLAQQRLLS 960
DRKDVVVLVI LRPDAHRSRY VRLRQRLCRQ SVLFWPQQPN GQGGFWAQLS TALTRDNRHF 1020
YNQNFRCRGPT AE 1032

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Since NF- κ B activation is central to the IL-1/TLR signal transduction pathway
 15 (Medzhitov R et al. (1998) *Mol Cell* 2:253-258 (1998); Muzio M et al. (1998) *J Exp Med* 187:2097-101), cells were transfected with hTLR9 or co-transfected with hTLR9 and an NF- κ B-driven luciferase reporter construct. Human 293 fibroblast cells were transiently transfected with (Figure 1A) hTLR9 and a six-times NF- κ B-luciferase reporter plasmid (NF- κ B-luc, kindly provided by Patrick Baeuerle, Munich, Germany)
 20 or (Figure 1B) with hTLR9 alone. After stimulus with CpG-ODN (2006, 2 μ M, TCGTCGTTTTGTCGTTTTGTCGTT, SEQ ID NO:15), GpC-ODN (2006-GC, 2 μ M, TGCTGCTTTTGTGCTTTTGTGCTT, SEQ ID NO:16), LPS (100 ng/ml) or media, NF- κ B activation by luciferase readout (8h, Figure 1A) or IL-8 production by ELISA (48h, Figure 1B) were monitored. Results are representative of three independent
 25 experiments. Figure 1 shows that cells expressing hTLR9 responded to CpG-DNA but not to LPS.

Figure 2 demonstrates the same principle for the transfection of mTLR9. Human 293 fibroblast cells were transiently transfected with mTLR9 and the NF- κ B-luc construct (Figure 2). Similar data was obtained for IL-8 production (not shown).
 30 Thus expression of TLR9 (human or mouse) in 293 cells results in a gain of function for CpG-DNA stimulation similar to hTLR4 reconstitution of LPS responses.

To generate stable clones expressing human TLR9, murine TLR9, or either TLR9 with the NF- κ B-luc reporter plasmid, 293 cells were transfected in 10 cm plates (2x10⁶ cells/plate) with 16 μ g of DNA and selected with 0.7 mg/ml G418 (PAA
 35 Laboratories GmbH, Cölbe, Germany). Clones were tested for TLR9 expression by RT-PCR, for example as shown in Figure 3. The clones were also screened for IL-8

production or NF- κ B-luciferase activity after stimulation with ODN. Four different types of clones were generated.

293-hTLR9-luc:	expressing human TLR9 and 6-fold NF- κ B-luciferase reporter
5 293-mTLR9-luc:	expressing murine TLR9 and 6-fold NF- κ B-luciferase reporter
293-hTLR9:	expressing human TLR9
293-mTLR9:	expressing murine TLR9

Figure 4 demonstrates the responsiveness of a stable 293-hTLR9-luc clone after
 10 stimulation with CpG-ODN (2006, 2 μ M), GpC-ODN (2006-GC, 2 μ M), Me-CpG-ODN
 (2006 methylated, 2 μ M; TZGTZGTTTTGTZGTTTTGTZGTT, Z = 5-methylcytidine,
 SEQ ID NO:17), LPS (100 ng/ml) or media, as measured by monitoring NF- κ B
 activation. Similar results were obtained utilizing IL-8 production with the stable clone
 293-hTLR9. 293-mTLR9-luc were also stimulated with CpG-ODN (1668, 2 μ M;
 15 TCCATGACGTTTCCTGATGCT, SEQ ID NO:18), GpC-ODN (1668-GC, 2 μ M;
 TCCATGAGCTTCCTGATGCT, SEQ ID NO:19), Me-CpG-ODN (1668 methylated,
 2 μ M; TCCATGAZGTTTCCTGATGCT, Z = 5-methylcytidine, SEQ ID NO:20), LPS
 (100 ng/ml) or media, as measured by monitoring NF- κ B activation (**Figure 5**).
 Similar results were obtained utilizing IL-8 production with the stable clone 293-
 20 mTLR9. Results are representative of at least two independent experiments. These
 results demonstrate that CpG-DNA non-responsive cell lines can be stably genetically
 complemented with TLR9 to become responsive to CpG-DNA in a motif-specific
 manner. These cells can be used for screening of optimal ligands for innate immune
 responses driven by TLR9 in multiple species.

25

Example 11. Reconstitution of TLR3 Signaling in 293 Fibroblasts

Human TLR3 cDNA and murine TLR3 cDNA in pT-Adv vector (from
 Clontech) were individually cloned into the expression vector pcDNA3.1(-) from
 Invitrogen using the EcoRI site. The resulting expression vectors mentioned above
 30 were transfected into CpG-DNA non-responsive human 293 fibroblast cells (ATCC,
 CRL-1573) using the calcium phosphate method. Utilizing a "gain of function" assay it

was possible to reconstitute human TLR3 (hTLR3) and murine TLR3 (mTLR3) signaling in 293 fibroblast cells.

Since NF- κ B activation is central to the IL-1/TLR signal transduction pathway (Medzhitov R et al. (1998) *Mol Cell* 2:253-8; Muzio M et al. (1998) *J Exp Med* 187:2097-101), in a first set of experiments human 293 fibroblast cells were transfected with hTLR3 alone or co-transfected with hTLR3 and an NF- κ B-driven luciferase reporter construct.

Likewise, in a second set of experiments, 293 fibroblast cells were transfected with hTLR3 alone or co-transfected with hTLR3 and an IFN- α 4-driven luciferase reporter construct (described in Example 2 above).

In a third group of experiments, 293 fibroblast cells were transfected with hTLR3 alone or co-transfected with hTLR3 and a RANTES-driven luciferase reporter construct (described in Example 5 above).

15 **Example 12. Proline to Histidine Mutation P915H in the TIR Domain of Human and Murine TLR9 Alters TLR9 Signaling**

Toll-like receptors have a cytoplasmic Toll/IL-1 receptor (TIR) homology domain which initiates signaling after binding of the adapter molecule MyD88. Medzhitov R et al. (1998) *Mol Cell* 2:253-8; Kopp EB et al. (1999) *Curr Opin Immunol* 11:15-8. Reports by others have shown that a single point mutation in the signaling TIR domain in murine TLR4 (Pro712 to His, P712H) or human TLR2 (Pro681 to His, P681H) abolishes host immune response to lipopolysaccharide or gram-positive bacteria, respectively. Poltorak A et al. (1998) *Science* 282:2085-8; Underhill DM et al. (1999) *Nature* 401:811-5. Through site-specific mutagenesis the equivalent proline (P) at position 915 of human TLR9 and murine TLR9 were mutated to histidine (H; P915H). These mutations were generated by the use of the primers 5'-GCGACTGGCTGCATGGCAAACCCCTCTTTG-3' (SEQ ID NO:21) and 5'-CAAAGAGGGTTTTGCCATGCAGCCAGTCGC-3' (SEQ ID NO:22) for human TLR9 and the primers 5'-CGAGATTGGCTGCATGGCCAGACGCTCTTC-3' (SEQ ID NO:23) and 5'-GAAGAGCGTCTGGCCATGCAGCCAATCTCG-3' (SEQ ID NO:24) for murine TLR9. Expression vectors for the mutant TLR9s, hTLR9-P915H

and mTLR9-P915H, were constructed and verified using standard recombinant DNA techniques.

For the stimulation of human TLR9 variant, hTLR9-P915H, 293 cells were transiently transfected with expression vector for hTLR9 or hTLR9-P915H and
5 stimulated after 16 hours with ODN 2006 or ODN 1668 at various concentrations. Likewise for the stimulation of murine TLR9 variant, mTLR9-P915H, 293 cells were transiently transfected with expression vector for mTLR9 or mTLR9-P915H and stimulated after 16 hours with ODN 2006 or ODN 1668 at various concentrations. After 48 hours of stimulation, supernatant was harvested and IL-8 production was
10 measured by ELISA. Results demonstrated that TLR9 activity can be destroyed by the P915H mutation in the TIR domain of both human and murine TLR9.

Example 13. Exchange of the TIR Domain Between Human TLR3 and Human TLR9 (hTLR3-TIR9 and hTLR9-TIR3)

15 While TLR3 and TLR9 share many structural features, TLR3, by virtue of its having an alanine rather than proline at a critical position in the TIR domain, may not be able to signal via MyD88 as does TLR9. The chimeric TLRs described here can be used in the screening assays of the invention. To generate molecules consisting of human extracellular TLR3 and the TIR domain of human TLR9 (hTLR3-TIR9), the
20 following approach can be used. Through site-specific mutagenesis a ClaI restriction site is introduced in human TLR3 and human TLR9. For human TLR9 the DNA sequence 5'-GGCCTCAGCATCTTT-3' (3026-3040, SEQ ID NO:25) is mutated to 5'-GGCCTATCGATTTT-3' (SEQ ID NO:26), introducing a ClaI site (underlined in the sequence) but leaving the amino acid sequence (GLSIF, aa 798-802) unchanged. For
25 human TLR3 the DNA sequence 5'-GGGTTCCCAGTGAGA-3' (2112-2126, SEQ ID NO:27) is mutated to 5'-GGGTTATCGATTAGA-3' (SEQ ID NO:28), introducing a ClaI site and creating the amino acid sequence (GLSIR, aa 685-689) which differs in three positions (aa 686, 687, 688) from the wildtype human TLR3 sequence (GFPVR, aa 685-689).

30 **hTLR3-TIR9.** The primers used for human TLR9 are 5'-CAGCTCCAGGGCCTATCGATTTTGCACAGGACC-3' (SEQ ID NO:29) and 5'-GGTCCTGTGCAAAAATCGATAGGCCCTGGAGCTG-3' (SEQ ID NO:30). For

creating an expression vector containing the extracellular portion of human TLR3 connected to the TIR domain of human TLR9, the human TLR3 expression vector is cut with ClaI and limiting amounts of EcoRI and the fragment coding for the TIR domain of human TLR9 generated by a ClaI and EcoRI digestion of human TLR9 expression vector is ligated in the vector fragment containing the extracellular portion of hTLR3. Transfection into *E.coli* yields the expression vector hTLR3-TIR9 (human extracellular TLR3-human TLR9 TIR domain). The expressed product of hTLR3-TIR9 can interact with TLR3 ligands and also signal through an MyD88-mediated signal transduction pathway.

hTLR9-TIR3. A fusion construct with the extracellular domain of hTLR9 and the TIR domain of hTLR3 is prepared using an analogous strategy. For creating an expression vector containing the extracellular portion of human TLR9 connected to the TIR domain of human TLR3, the human TLR9 expression vector is cut with ClaI and limiting amounts of EcoRI and the fragment coding for the TIR domain of human TLR3 generated by a ClaI and EcoRI digestion of human TLR3 expression vector is ligated in the vector fragment containing the extracellular portion of hTLR9. Transfection into *E.coli* yields the expression vector hTLR9-TIR3 (human extracellular TLR9-human TLR3 TIR domain). The expressed product of hTLR9-TIR3 can interact with TLR9 ligands, e.g., CpG DNA, and signal through a signal transduction pathway in a manner like TLR3.

Example 14. Sensitive in vitro Assay for Detecting Ligand Affinity Differences for a TLR

Human 293 fibroblast cells stably transfected with murine TLR9 and an NF- κ B-luciferase reporter were stimulated for 16 hours with the following fully phosphorothioated oligodeoxynucleotides (ODN):

5890: T*C*C*A*T*G*A*C*G*T*T*T*T*G*A*T*G*T*T (SEQ ID NO:31)
 5895: T*C*C*A*T*G*A*C*G*T*T*T*T*G*A*T*G (SEQ ID NO:32)
 5896: T*C*C*A*T*G*A*C*G*T*T*T*T*G*A (SEQ ID NO:33)
 5897: T*C*C*A*T*G*A*C*G*T*T*T*T (SEQ ID NO:34)

Concentration of the stimulus was titrated between 10 μ M and 2 nM. The data is plotted in Figure 6 as fold induction of NF- κ B luciferase, relative to unstimulated

background, versus ODN concentration. The data displays typical first-order binding curves from which EC50 or maximal activity can be determined. EC50 is defined as the concentration of the ligand stimulus that results in 50% maximal activation. As shown in the figure, the EC50 ranges from 42 nM for ODN 5890 to 1220 nM for ODN 5897. The assay demonstrates sensitive differentiation between subtle changes in ligand.

Example 15. Influence of Assay Kinetics on TLR Screening Assays

Curves were prepared as in the previous Example 14 with the following ODN ligands, where * indicates phosphorothioate and _ indicates phosphodiester linkage:

ODN 5890: T*G*C*A*T*G*A*C*G*T*T*T*T*G*A*T*G*T*T	(SEQ ID NO:35)
ODN 5891: T*G*C*G*T*C*G*T*T*T*T_G_T_C_G_T*T*T*G*T*C*G*T*T	(SEQ ID NO:36)
ODN 5892: T*G*C_G*T*C_G*T*T*T*T_G*T*C_G*T*T*T*G*T*C_G*T*T	(SEQ ID NO:37)
ODN 2006: T*G*C*G*T*C*G*T*T*T*T*G*T*C*G*T*T*T*G*T*C*G*T*T	(SEQ ID NO:15)
ODN 5897: T*G*C*A*T*G*A*C_G_T*T*T*T*G*A*T_G*T*T	(SEQ ID NO:38)

A family of stimulation curves was determined at various times of assay incubation between 1 and 24 hours. The EC50 was determined for each ligand at each time point. The EC50 was then plotted versus time to yield the resultant curves shown in Figure 7.

As evident from Figure 7, it is demonstrated that the kinetics of activation vary dependent on the ligand tested. Because luciferase has a three-hour half-life, the signal is transient and requires constant promoter-driven activation to be maintained. The response is directly related to the signal delivered by the ligand/receptor complex. This analysis of time kinetics in such a fashion allows one to determine both affinity of ligand/receptor interaction and the availability of the ligand to the receptor through time. The principle is demonstrated as follows. The ODN 5890 is of higher affinity compared to the ODN 2006. When the ligand is made more labile to destruction by incorporating less stable diester linkages, the activity curves turn upward with time such as for ODN 5746, 5902 and 5497.

In the context of a screening assay for TLR/ligand interactions, limiting the assay to one time point would bias the assay. At 24 hours it would appear that only ODN 2006 and 5890 were ligand candidates, however this is clearly not the case. The assay also demonstrates that earlier time points, such as 6 hours in this example, would be an optimal time point for determining the greatest difference between

receptor/ligand affinities. Thus optimization of the screening assay can be adjusted depending on the desired information to be obtained from the screen, e.g., higher affinity of interaction versus stability and duration of receptor/ligand interaction.

Figure 8 demonstrates the same principles shown with a murine TLR as in this example can be applied independent of the TLR utilized. For this set of data a 293 cell stably transfected with human TLR9 and NF- κ B-luciferase was used.

Example 16. Influence of Assay Kinetics on Maximal Activities in TLR Screening Assays

Data was collected as in the previous Example 15, however the maximal activity (maximal fold induction) was plotted versus time in Figures 9 and 10. Such data analysis results in a prediction of biological efficacy. As can be seen from these figures, the lower affinity ODN, e.g., ODN 2006 and 5890 as demonstrated by the EC50 curves of Example 15, are clearly less efficient at delivering high activity.

Example 17. Differential Outcomes of TLR Screening Assays Dependent on Promoter Utilization

Human 293 fibroblast cells were transiently transfected with expression vector for TLR 7, TLR8, or TLR9 and one of the following reporter constructs bearing the following promoters driving the luciferase gene: NF- κ B-luc, IP-10-luc, RANTES-luc, ISRE-luc, and IL-8-luc. The cells were stimulated for 16h with the maximal activity concentration of specific ligand. TLR9 was stimulated with CpG ODN 2006; TLR8 and TLR7 were stimulated with the imidazoliquinalone R848. Results are shown in Figure 11. As evident from the figure, the promoter used influences the outcome of the screening assay dependent on the TLR in question. For example, NF- κ B is a reliable marker for all TLRs tested, whereas in this set of experiments ISRE was only functional to some extent for TLR8. The IL-8 promoter is particularly sensitive for TLR7 or TLR8 screening assays but would be much less efficient in TLR9 assays.

What is claimed is:

Claims

1. A screening method for identifying an immunostimulatory compound,
comprising:
5 contacting a functional TLR3 with a test compound under conditions which, in
absence of the test compound, permit a negative control response mediated by a
TLR3 signal transduction pathway;
 detecting a test response mediated by the TLR3 signal transduction pathway;
and
10 determining the test compound is an immunostimulatory compound when the
test response exceeds the negative control response.
2. A screening method for identifying an immunostimulatory compound,
comprising:
15 contacting a functional TLR3 with a test compound under conditions which, in
presence of a reference immunostimulatory compound, permit a reference response
mediated by a TLR3 signal transduction pathway;
 detecting a test response mediated by the TLR3 signal transduction pathway;
and
20 determining the test compound is an immunostimulatory compound when the
test response equals or exceeds the reference response.
3. A screening method for identifying a compound that modulates TLR3 signaling
activity, comprising:
25 contacting a functional TLR3 with a test compound and a reference
immunostimulatory compound under conditions which, in presence of the reference
immunostimulatory compound alone, permit a reference response mediated by a
TLR3 signal transduction pathway;
 detecting a test-reference response mediated by the TLR3 signal transduction
30 pathway;
 determining the test compound is an agonist of TLR3 signaling activity when
the test-reference response exceeds the reference response; and

determining the test compound is an antagonist of TLR3 signaling activity when the reference response exceeds the test-reference response.

4. A screening method for identifying species specificity of an immunostimulatory compound, comprising:
- 5 measuring a first species-specific response mediated by a TLR3 signal transduction pathway when a functional TLR3 of a first species is contacted with a test compound;
- 10 measuring a second species-specific response mediated by the TLR3 signal transduction pathway when a functional TLR3 of a second species is contacted with the test compound; and
- comparing the first species-specific response with the second species-specific response.
- 15 5. The method of any one of claims 1-4, wherein the screening method is performed on a plurality of test compounds.
6. The method of claim 5, wherein the response mediated by the TLR3 signal transduction pathway is measured quantitatively.
- 20 7. The method of any one of claims 1-4, wherein the functional TLR3 is expressed in a cell.
8. The method of claim 7, wherein the cell is an isolated mammalian cell that
- 25 naturally expresses the functional TLR3.
9. The method of claim 7, wherein the cell is an isolated mammalian cell that does not naturally express the functional TLR3, and wherein the cell comprises an expression vector for TLR3.
- 30 10. The method of claim 9, wherein the cell is a 293 human fibroblast.

11. The method of claim 7, wherein the cell comprises an expression vector comprising an isolated nucleic acid which encodes a reporter construct selected from the group of interleukin-6-luciferase (IL-6-luc), IL-8-luc, IL-12 p40-luc, IL-12 p40- β -Gal, NF- κ B-luc, AP1-luc, IFN- α -luc, IFN- β -luc, RANTES-luc, TNF-luc, IP-10-luc, I-TAC-luc, and ISRE-luc.
12. The method of claim 11, wherein the reporter construct is ISRE-luc.
13. The method of any one of claims 1-4, wherein the functional TLR3 is part of a cell-free system.
14. The method of any one of claims 1-4, wherein the functional TLR3 is part of a complex with a non-TLR protein selected from the group consisting of MyD88, IL-1 receptor associated kinase 1-3 (IRAK1, IRAK2, IRAK3), tumor necrosis factor receptor-associated factor 1-6 (TRAF1 - TRAF6), I κ B, NF- κ B, MyD88-adaptor-like (Mal), Toll-interleukin 1 receptor (TIR) domain-containing adapter protein (TIRAP), Tollip, Rac, and functional homologues and derivatives thereof.
15. The method of claim 14, wherein the non-TLR protein excludes MyD88.
16. The method of claim 2 or 3, wherein the reference immunostimulatory compound is a nucleic acid.
17. The method of claim 16, wherein the nucleic acid is a CpG nucleic acid.
18. The method of claim 2 or 3, wherein the reference immunostimulatory compound is a small molecule.
19. The method of any one of claims 1-4, wherein the test compound is a part of a combinatorial library of compounds.

20. The method of any one of claims 1-4, wherein the test compound is a nucleic acid.
21. The method of claim 20, wherein the nucleic acid is a CpG nucleic acid.
- 5 22. The method of any one of claims 1-4, wherein the test compound is a small molecule.
- 10 23. The method of any one of claims 1-4, wherein the test compound is a polypeptide.
24. The method of any one of claims 1-4, wherein the response mediated by a TLR3 signal transduction pathway is induction of a reporter gene under control of a promoter response element selected from the group consisting of ISRE, IL-6, IL-8,
15 IL-12 p40, IFN- α , IFN- β , IFN- ω , RANTES, TNF, IP-10, and I-TAC.
25. The method of claim 24, wherein the reporter gene under control of a promoter response element is selected from the group consisting of ISRE-luc, IL-6-luc, IL-8-luc, IL-12 p40-luc, IL-12 p40- β -Gal, IFN- α -luc, IFN- β -luc, RANTES-luc, TNF-luc,
20 luc, IP-10-luc, and I-TAC-luc.
26. The method of claim 25, wherein the reporter gene under control of a promoter response element is ISRE-luc.
- 25 27. The method of claim 24, wherein the reporter gene is selected from the group consisting of IFN- α 1-luc and IFN- α 4-luc.
28. The method of any one of claims 1-4, wherein the response mediated by a TLR3 signal transduction pathway is selected from the group consisting of (a)
30 induction of a reporter gene under control of a minimal promoter responsive to a transcription factor selected from the group consisting of AP1, NF- κ B, ATF2, IRF3, and IRF7; (b) secretion of a chemokine; and (c) secretion of a cytokine.

29. The method of claim 28, wherein the response mediated by a TLR3 signal transduction pathway is induction of a reporter gene selected from the group consisting of AP1-luc and NF- κ B-luc.
- 5
30. The method of claim 28, wherein the response mediated by a TLR3 signal transduction pathway is secretion of a type 1 IFN.
31. The method of claim 28, wherein the response mediated by a TLR3 signal
10 transduction pathway is secretion of a chemokine selected from the group consisting of CCL5 (RANTES), CXCL9 (Mig), CXCL10 (IP-10), and CXCL11 (I-TAC).
32. The method of any one of claims 1-3, wherein the contacting a functional TLR3
15 with a test compound further comprises, for each test compound, contacting with the test compound at each of a plurality of concentrations.
33. The method of any one of claims 1-3, wherein the detecting is performed 6-12 hours following the contacting.
- 20
34. The method of any one of claims 1-3, wherein the detecting is performed 16-24 hours following the contacting.

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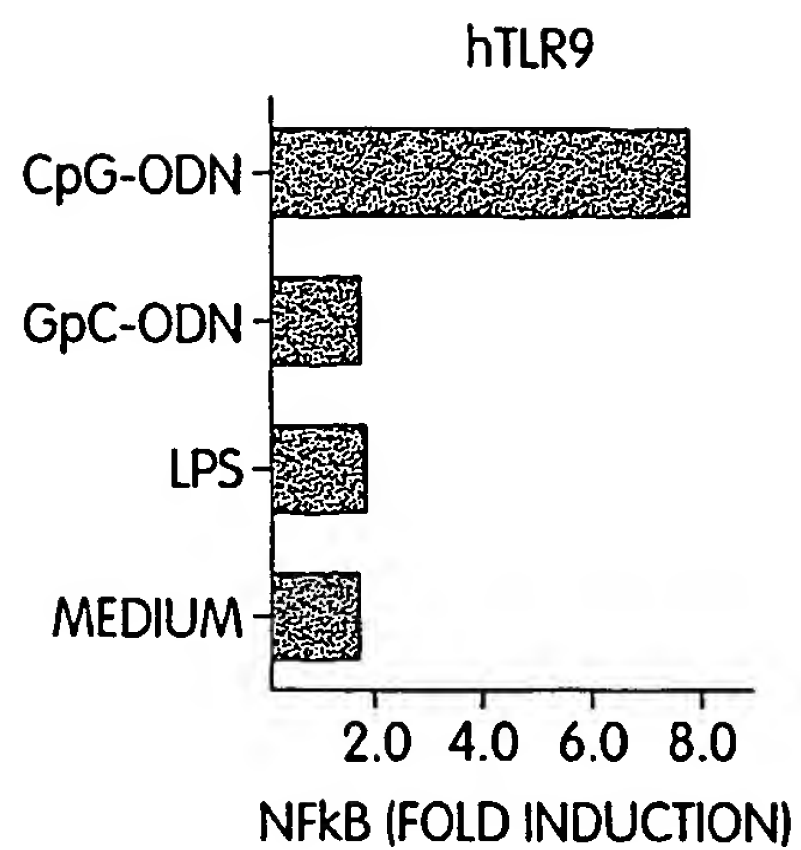


Fig. 1A

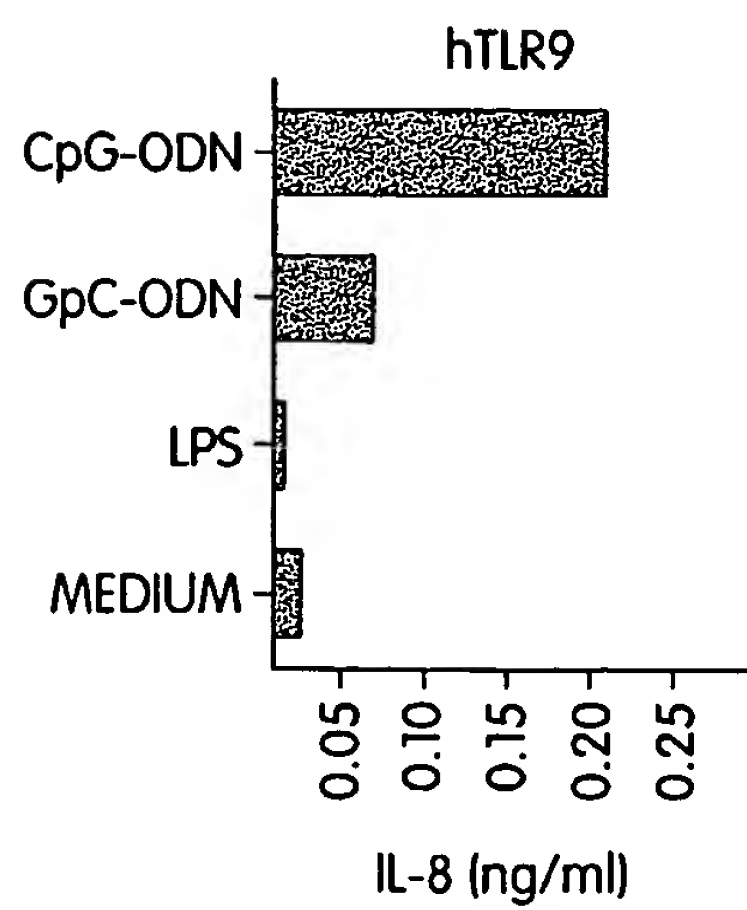


Fig. 1B

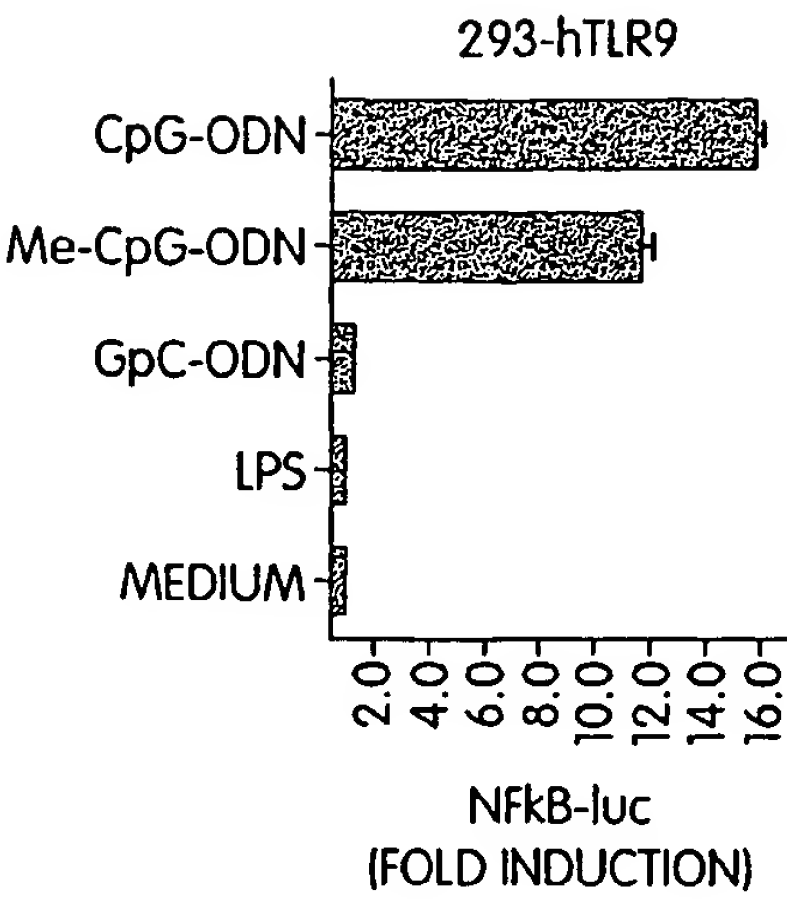


Fig. 2

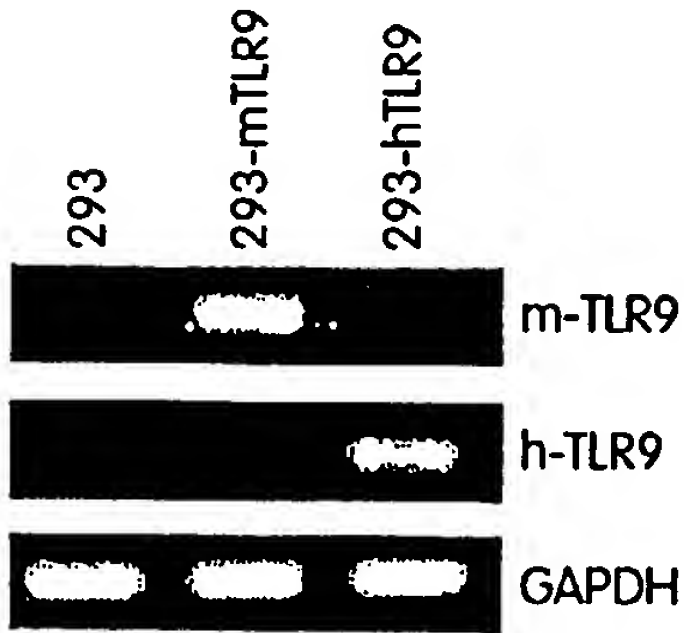


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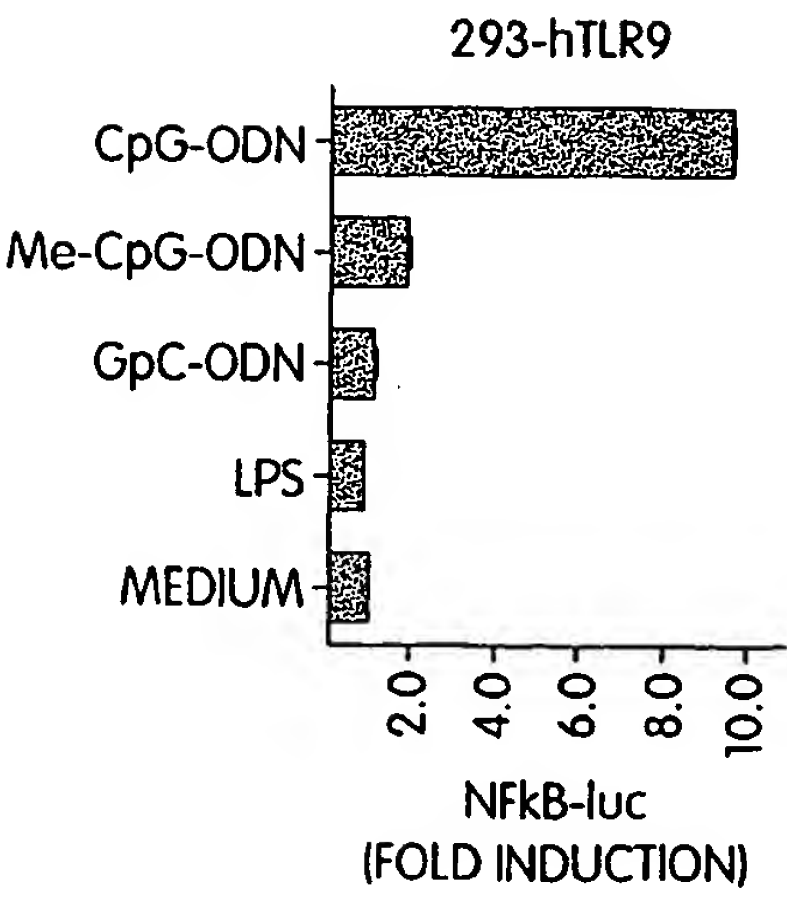


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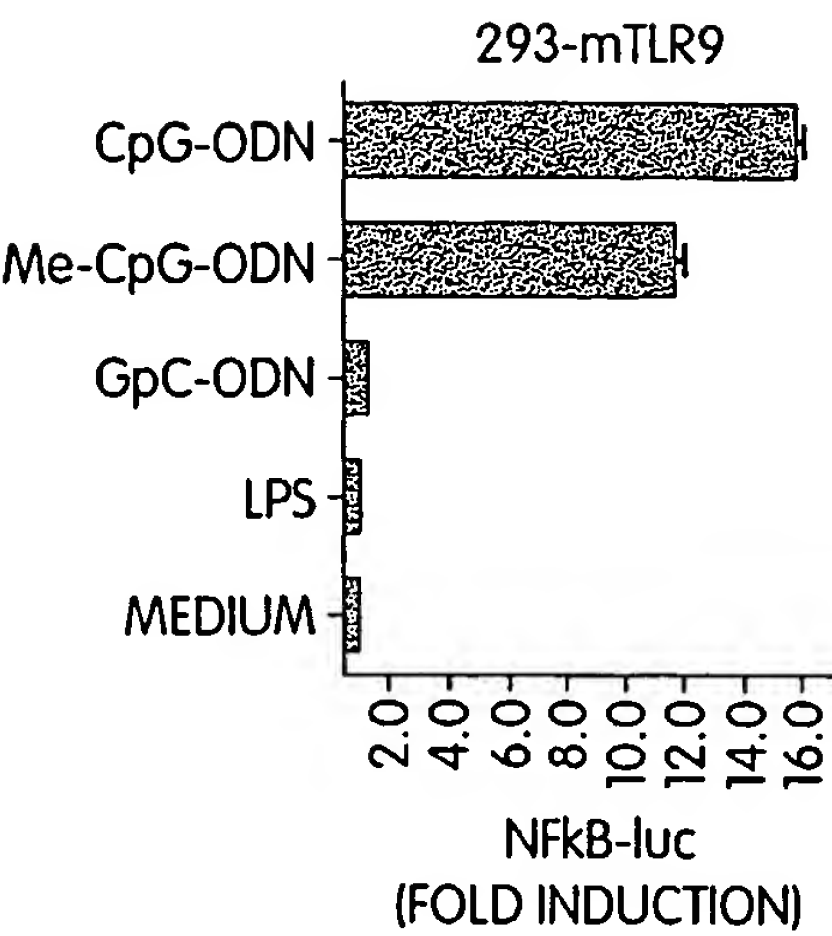


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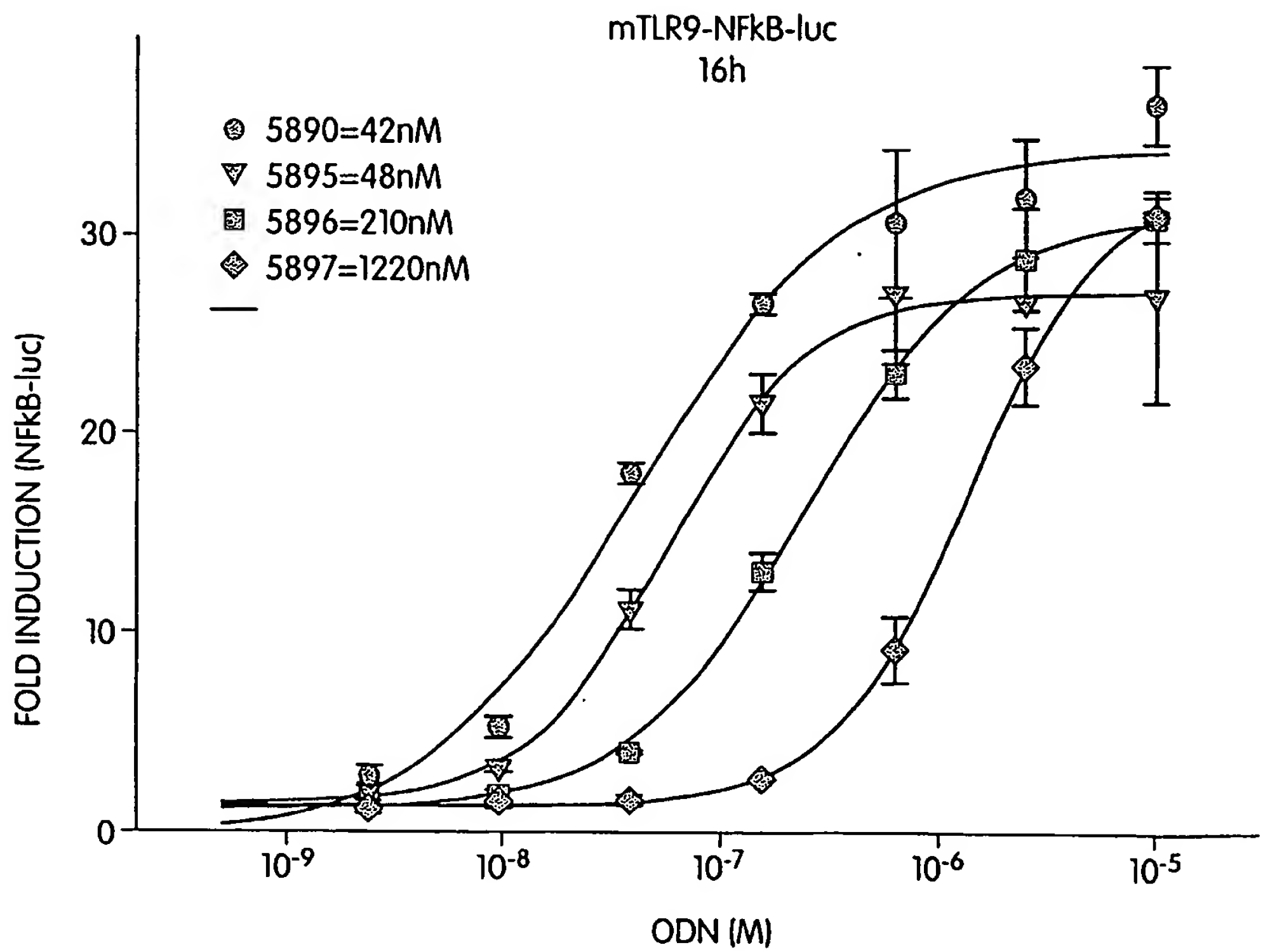


Fig. 6

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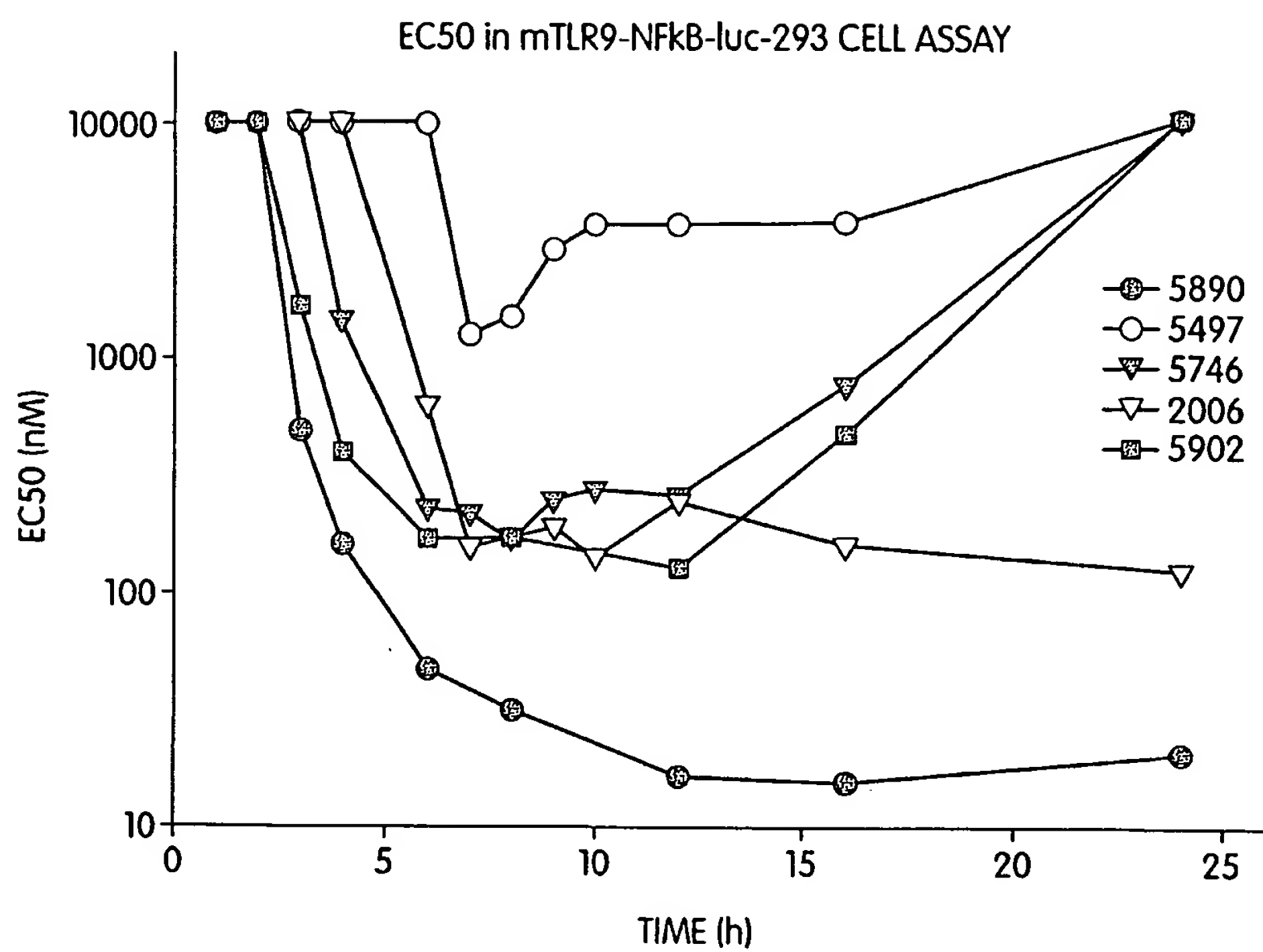


Fig. 7

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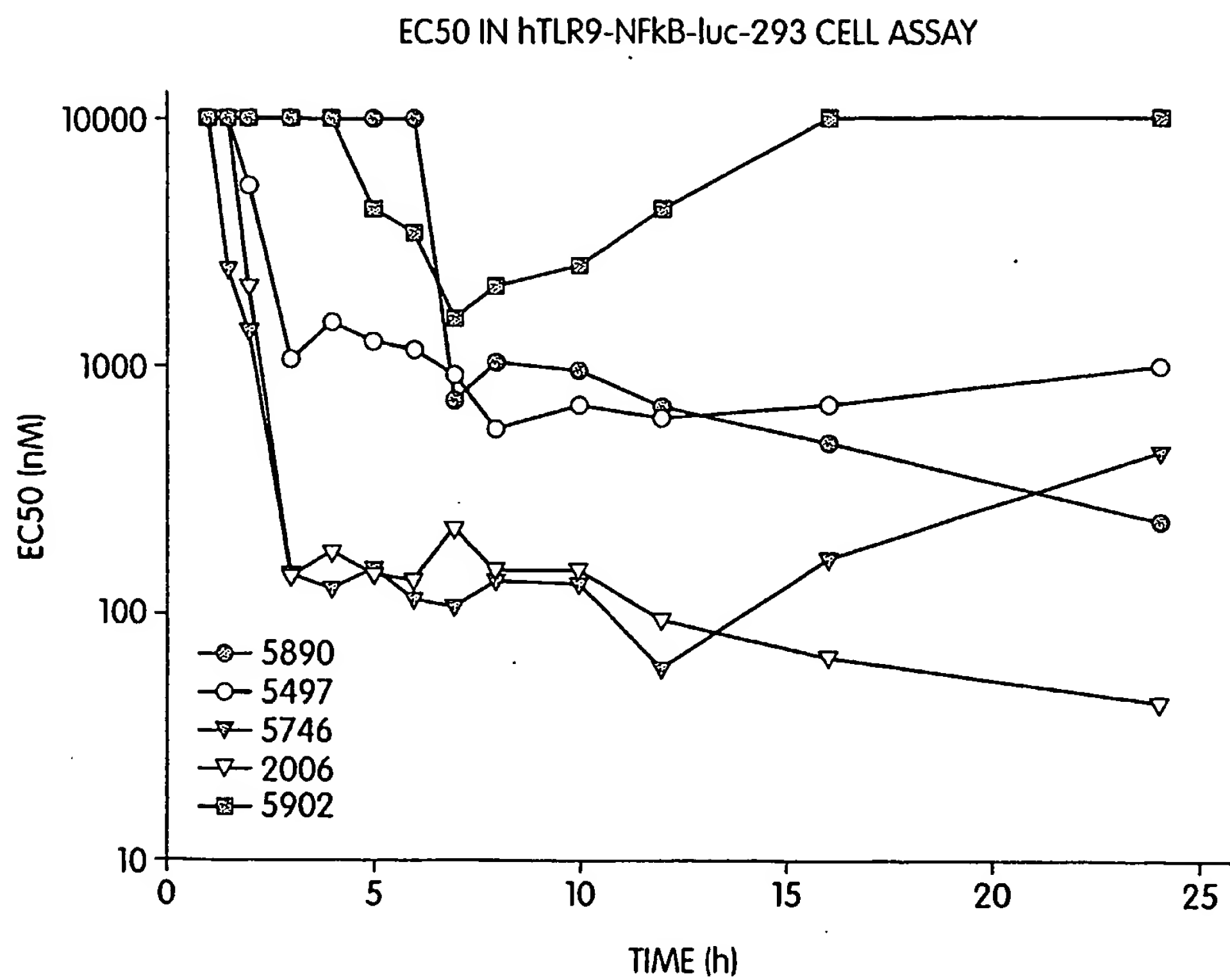


Fig. 8

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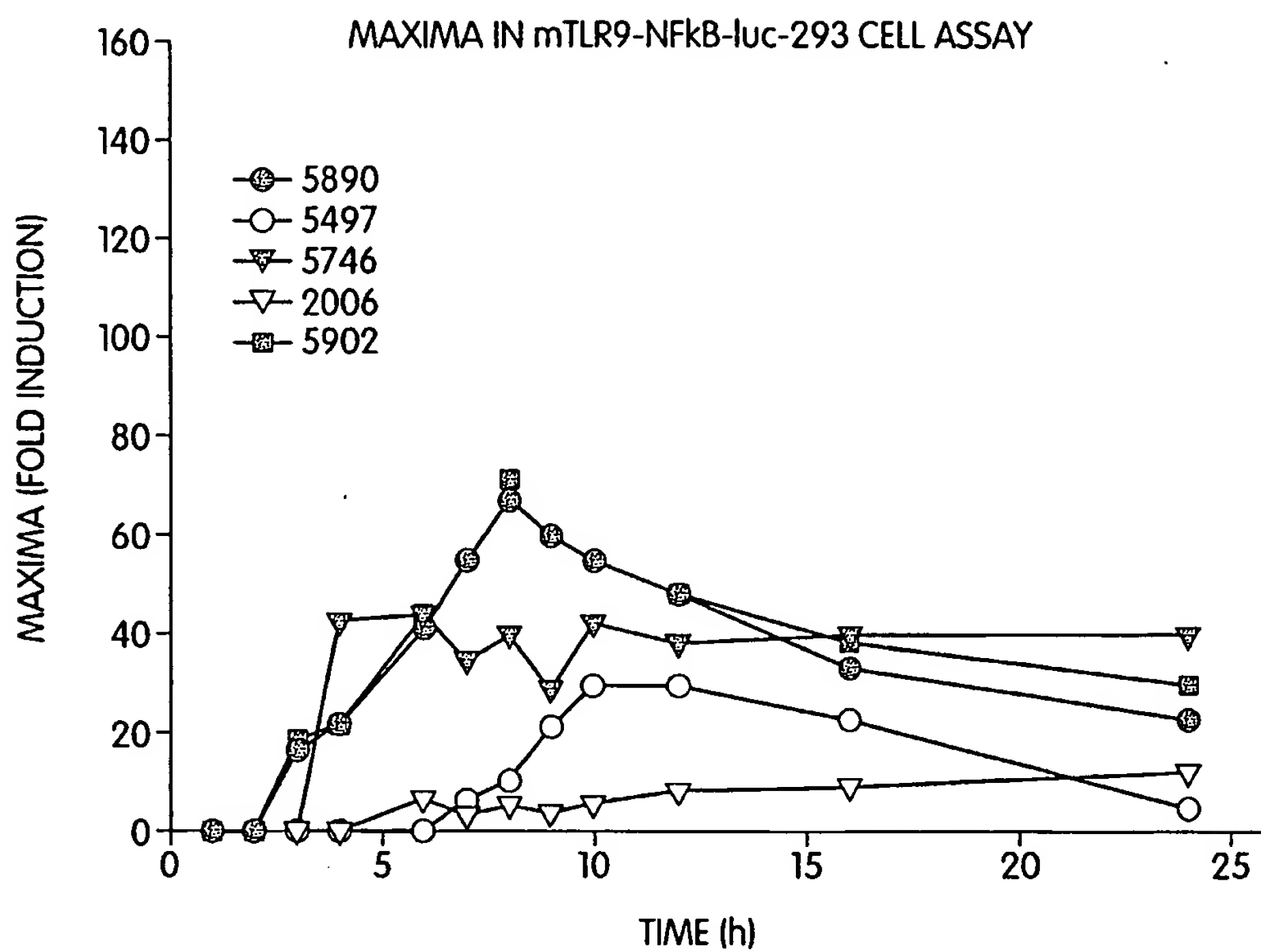


Fig. 9

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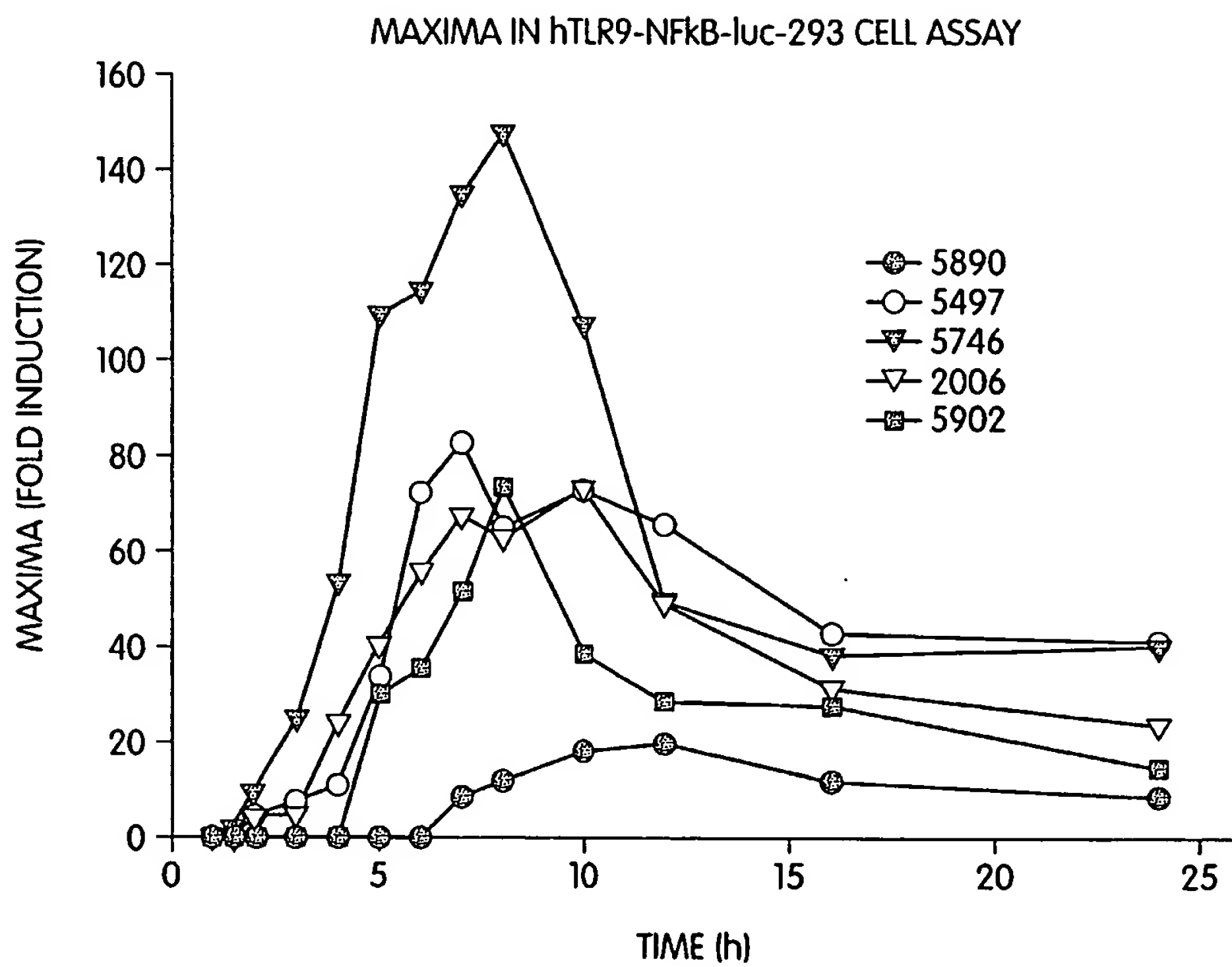


Fig. 10

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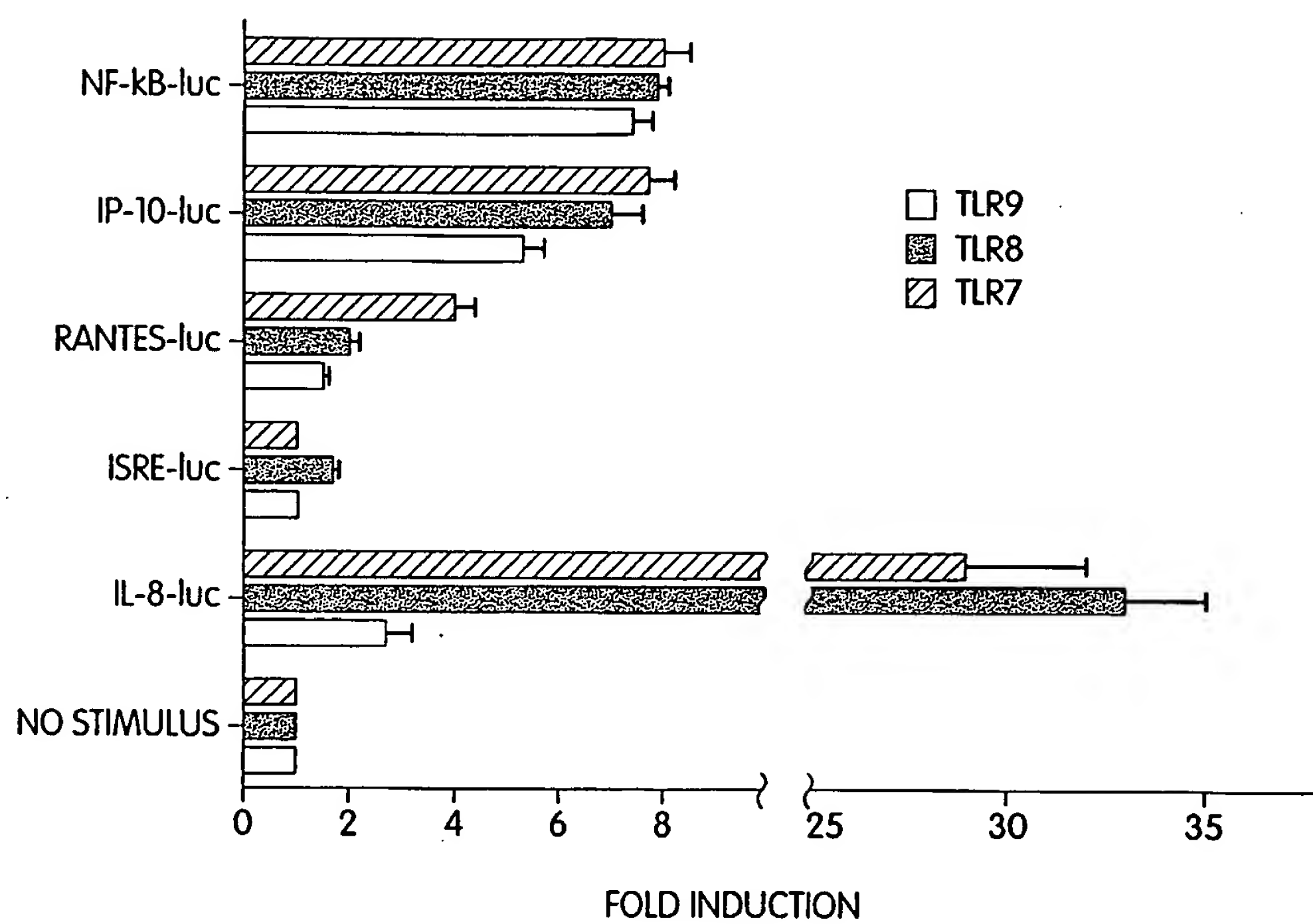


Fig. 11

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3029

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Phe Gly Met Leu Cys Ala Ser Ser Thr Thr Lys Cys Thr Val Ser His
20 25 30

Glu Val Ala Asp Cys Ser His Leu Lys Leu Thr Gln Val Pro Asp Asp
35 40 45

Leu Pro Thr Asn Ile Thr Val Leu Asn Leu Thr His Asn Gln Leu Arg
50 55 60

Arg Leu Pro Ala Ala Asn Phe Thr Arg Tyr Ser Gln Leu Thr Ser Leu
65 70 75 80

Asp Val Gly Phe Asn Thr Ile Ser Lys Leu Glu Pro Glu Leu Cys Gln
85 90 95

Lys Leu Pro Met Leu Lys Val Leu Asn Leu Gln His Asn Glu Leu Ser
100 105 110

Gln Leu Ser Asp Lys Thr Phe Ala Phe Cys Thr Asn Leu Thr Glu Leu
115 120 125

His Leu Met Ser Asn Ser Ile Gln Lys Ile Lys Asn Asn Pro Phe Val
130 135 140

Lys Gln Lys Asn Leu Ile Thr Leu Asp Leu Ser His Asn Gly Leu Ser
145 150 155 160

Ser Thr Lys Leu Gly Thr Gln Val Gln Leu Glu Asn Leu Gln Glu Leu
165 170 175

Leu Leu Ser Asn Asn Lys Ile Gln Ala Leu Lys Ser Glu Glu Leu Asp
180 185 190

Ile Phe Ala Asn Ser Ser Leu Lys Lys Leu Glu Leu Ser Ser Asn Gln

195	200	205
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Asn Ser Gln Leu Ser Thr Thr Ser Asn Thr Thr Phe Leu Gly Leu Lys 260 265 270		
Trp Thr Asn Leu Thr Met Leu Asp Leu Ser Tyr Asn Asn Leu Asn Val 275 280 285		
Val Gly Asn Asp Ser Phe Ala Trp Leu Pro Gln Leu Glu Tyr Phe Phe 290 295 300		
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Leu Phe Asn Val Arg Tyr Leu Asn Leu Lys Arg Ser Phe Thr Lys Gln 325 330 335		
Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile Asp Asp Phe Ser Phe Gln 340 345 350		
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Gly Ile Lys Ser Asn Met Phe Thr Gly Leu Ile Asn Leu Lys Tyr Leu 370 375 380		
Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg Thr Leu Thr Asn Glu Thr 385 390 395 400		
Phe Val Ser Leu Ala His Ser Pro Leu His Ile Leu Asn Leu Thr Lys 405 410 415		
Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala Phe Ser Trp Leu Gly His 420 425 430		

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Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile Phe Glu Ile Tyr Leu Ser
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Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn Ser Phe Ala Leu Val Pro
 465 470 475 480

Ser Leu Gln Arg Leu Met Leu Arg Arg Val Ala Leu Lys Asn Val Asp
 485 490 495

Ser Ser Pro Ser Pro Phe Gln Pro Leu Arg Asn Leu Thr Ile Leu Asp
 500 505 510

Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn Asp Asp Met Leu Glu Gly
 515 520 525

Leu Glu Lys Leu Glu Ile Leu Asp Leu Gln His Asn Asn Leu Ala Arg
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Leu Trp Lys His Ala Asn Pro Gly Gly Pro Ile Tyr Phe Leu Lys Gly
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Leu Ser His Leu His Ile Leu Asn Leu Glu Ser Asn Gly Phe Asp Glu
 565 570 575

Ile Pro Val Glu Val Phe Lys Asp Leu Phe Glu Leu Lys Ile Ile Asp
 580 585 590

Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro Ala Ser Val Phe Asn Asn
 595 600 605

Gln Val Ser Leu Lys Ser Leu Asn Leu Gln Lys Asn Leu Ile Thr Ser
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Val Glu Lys Lys Val Phe Gly Pro Ala Phe Arg Asn Leu Thr Glu Leu
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Asp Met Arg Phe Asn Pro Phe Asp Cys Thr Cys Glu Ser Ile Ala Trp
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Phe Val Asn Trp Ile Asn Glu Thr His Thr Asn Ile Pro Glu Leu Ser
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Ser His Tyr Leu Cys Asn Thr Pro Pro His Tyr His Gly Phe Pro Val
 675 680 685

Arg Leu Phe Asp Thr Ser Ser Cys Lys Asp Ser Ala Pro Phe Glu Leu
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Phe Phe Met Ile Asn Thr Ser Ile Leu Leu Ile Phe Ile Phe Ile Val
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Leu Leu Ile His Phe Glu Gly Trp Arg Ile Ser Phe Tyr Trp Asn Val
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Ser Val His Arg Val Leu Gly Phe Lys Glu Ile Asp Arg Gln Thr Glu
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Gln Phe Glu Tyr Ala Ala Tyr Ile Ile His Ala Tyr Lys Asp Lys Asp
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Trp Val Trp Glu His Phe Ser Ser Met Glu Lys Glu Asp Gln Ser Leu
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Lys Phe Cys Leu Glu Glu Arg Asp Phe Glu Ala Gly Val Phe Glu Leu
 785 790 795 800

Glu Ala Ile Val Asn Ser Ile Lys Arg Ser Arg Lys Ile Ile Phe Val
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Ile Thr His His Leu Leu Lys Asp Pro Leu Cys Lys Arg Phe Lys Val
 820 825 830

His His Ala Val Gln Gln Ala Ile Glu Gln Asn Leu Asp Ser Ile Ile
 835 840 845

Leu Val Phe Leu Glu Glu Ile Pro Asp Tyr Lys Leu Asn His Ala Leu
 850 855 860

Cys Leu Arg Arg Gly Met Phe Lys Ser His Cys Ile Leu Asn Trp Pro
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Val Gln Lys Glu Arg Ile Gly Ala Phe Arg His Lys Leu Gln Val Ala
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Leu Gly Ser Lys Asn Ser Val His
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3310

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<213> Mus musculus

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Ser Leu Trp Ile Leu Leu Val Ser Ser Thr Asn Gln Cys Thr Val Arg
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Tyr Asn Val Ala Asp Cys Ser His Leu Lys Leu Thr His Ile Pro Asp
 35 40 45

Asp Leu Pro Ser Asn Ile Thr Val Leu Asn Leu Thr His Asn Gln Leu
 50 55 60

Arg Arg Leu Pro Pro Thr Asn Phe Thr Arg Tyr Ser Gln Leu Ala Ile
 65 70 75 80

Leu Asp Ala Gly Phe Asn Ser Ile Ser Lys Leu Glu Pro Glu Leu Cys
 85 90 95

Gln Ile Leu Pro Leu Leu Lys Val Leu Asn Leu Gln His Asn Glu Leu
 100 105 110

Ser Gln Ile Ser Asp Gln Thr Phe Val Phe Cys Thr Asn Leu Thr Glu
 115 120 125

Leu Asp Leu Met Ser Asn Ser Ile His Lys Ile Lys Ser Asn Pro Phe
 130 135 140

Lys Asn Gln Lys Asn Leu Ile Lys Leu Asp Leu Ser His Asn Gly Leu
 145 150 155 160

Ser Ser Thr Lys Leu Gly Thr Gly Val Gln Leu Glu Asn Leu Gln Glu
 165 170 175

Leu Leu Leu Ala Lys Asn Lys Ile Leu Ala Leu Arg Ser Glu Glu Leu
 180 185 190

Glu Phe Leu Gly Asn Ser Ser Leu Arg Lys Leu Asp Leu Ser Ser Asn

195	200	205
Pro Leu Lys Glu Phe Ser	Pro Gly Cys Phe Gln Thr	Ile Gly Lys Leu
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Phe Ala Leu Leu Leu Asn Asn Ala Gln Leu Asn Pro His Leu Thr Glu		
225	230	235 240
Lys Leu Cys Trp Glu Leu Ser Asn Thr Ser Ile Gln Asn Leu Ser Leu		
245	250	255
Ala Asn Asn Gln Leu Leu Ala Thr Ser Glu Ser Thr Phe Ser Gly Leu		
260	265	270
Lys Trp Thr Asn Leu Thr Gln Leu Asp Leu Ser Tyr Asn Asn Leu His		
275	280	285
Asp Val Gly Asn Gly Ser Phe Ser Tyr Leu Pro Ser Leu Arg Tyr Leu		
290	295	300
Ser Leu Glu Tyr Asn Asn Ile Gln Arg Leu Ser Pro Arg Ser Phe Tyr		
305	310	315 320
Gly Leu Ser Asn Leu Arg Tyr Leu Ser Leu Lys Arg Ala Phe Thr Lys		
325	330	335
Gln Ser Val Ser Leu Ala Ser His Pro Asn Ile Asp Asp Phe Ser Phe		
340	345	350
Gln Trp Leu Lys Tyr Leu Glu Tyr Leu Asn Met Asp Asp Asn Asn Ile		
355	360	365
Pro Ser Thr Lys Ser Asn Thr Phe Thr Gly Leu Val Ser Leu Lys Tyr		
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Leu Ser Leu Ser Lys Thr Phe Thr Ser Leu Gln Thr Leu Thr Asn Glu		
385	390	395 400
Thr Phe Val Ser Leu Ala His Ser Pro Leu Leu Thr Leu Asn Leu Thr		
405	410	415
Lys Asn His Ile Ser Lys Ile Ala Asn Gly Thr Phe Ser Trp Leu Gly		
420	425	430

Gln Leu Arg Ile Leu Asp Leu Gly Leu Asn Glu Ile Glu Gln Lys Leu
 435 440 445

Ser Gly Gln Glu Trp Arg Gly Leu Arg Asn Ile Phe Glu Ile Tyr Leu
 450 455 460

Ser Tyr Asn Lys Tyr Leu Gln Leu Ser Thr Ser Ser Phe Ala Leu Val
 465 470 475 480

Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val Ala Leu Lys Asn Val
 485 490 495

Asp Ile Ser Pro Ser Pro Phe Arg Pro Leu Arg Asn Leu Thr Ile Leu
 500 505 510

Asp Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn Glu Asp Leu Leu Glu
 515 520 525

Gly Leu Glu Asn Leu Glu Ile Leu Asp Phe Gln His Asn Asn Leu Ala
 530 535 540

Arg Leu Trp Lys Arg Ala Asn Pro Gly Gly Pro Val Asn Phe Leu Lys
 545 550 555 560

Gly Leu Ser His Leu His Ile Leu Asn Leu Glu Ser Asn Gly Leu Asp
 565 570 575

Glu Ile Pro Val Gly Val Phe Lys Asn Leu Phe Glu Leu Lys Ser Ile
 580 585 590

Asn Leu Gly Leu Asn Asn Leu Asn Lys Leu Glu Pro Phe Ile Phe Asp
 595 600 605

Asp Gln Thr Ser Leu Arg Ser Leu Asn Leu Gln Lys Asn Leu Ile Thr
 610 615 620

Ser Val Glu Lys Asp Val Phe Gly Pro Pro Phe Gln Asn Leu Asn Ser
 625 630 635 640

Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr Cys Glu Ser Ile Ser
 645 650 655

Trp Phe Val Asn Trp Ile Asn Gln Thr His Thr Asn Ile Phe Glu Leu
 660 665 670

Ser Thr His Tyr Leu Cys Asn Thr Pro His His Tyr Tyr Gly Phe Pro
 675 680 685

Leu Lys Leu Phe Asp Thr Ser Ser Cys Lys Asp Ser Ala Pro Phe Glu
 690 695 700

Leu Leu Phe Ile Ile Ser Thr Ser Met Leu Leu Val Phe Ile Leu Val
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Val Leu Leu Ile His Ile Glu Gly Trp Arg Ile Ser Phe Tyr Trp Asn
 725 730 735

Val Ser Val His Arg Ile Leu Gly Phe Lys Glu Ile Asp Thr Gln Ala
 740 745 750

Glu Gln Phe Glu Tyr Thr Ala Tyr Ile Ile His Ala His Lys Asp Arg
 755 760 765

Asp Trp Val Trp Glu His Phe Ser Pro Met Glu Glu Gln Asp Gln Ser
 770 775 780

Leu Lys Phe Cys Leu Glu Glu Arg Asp Phe Glu Ala Gly Val Leu Gly
 785 790 795 800

Leu Glu Ala Ile Val Asn Ser Ile Lys Arg Ser Arg Lys Ile Ile Phe
 805 810 815

Val Ile Thr His His Leu Leu Lys Asp Pro Leu Cys Arg Arg Phe Lys
 820 825 830

Val His His Ala Val Gln Gln Ala Ile Glu Gln Asn Leu Asp Ser Ile
 835 840 845

Ile Leu Ile Phe Leu Gln Asn Ile Pro Asp Tyr Lys Leu Asn His Ala
 850 855 860

Leu Cys Leu Arg Arg Gly Met Phe Lys Ser His Cys Ile Leu Asn Trp
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Pro Val Gln Lys Glu Arg Ile Asn Ala Phe His His Lys Leu Gln Val
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Ala Leu Gly Ser Arg Asn Ser Ala His
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 <212> DNA
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3352

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<212> PRT

<213> Homo sapiens

<400> 6

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Leu Pro Cys Glu Leu Gln Pro His Gly Leu Val Asn Cys Asn Trp Leu
35 40 45

Phe Leu Lys Ser Val Pro His Phe Ser Met Ala Ala Pro Arg Gly Asn
50 55 60

Val Thr Ser Leu Ser Leu Ser Ser Asn Arg Ile His His Leu His Asp
65 70 75 80

Ser Asp Phe Ala His Leu Pro Ser Leu Arg His Leu Asn Leu Lys Trp
85 90 95

Asn Cys Pro Pro Val Gly Leu Ser Pro Met His Phe Pro Cys His Met
100 105 110

Thr Ile Glu Pro Ser Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu
115 120 125

Asn Leu Ser Tyr Asn Asn Ile Met Thr Val Pro Ala Leu Pro Lys Ser
130 135 140

Leu Ile Ser Leu Ser Leu Ser His Thr Asn Ile Leu Met Leu Asp Ser
145 150 155 160

Ala Ser Leu Ala Gly Leu His Ala Leu Arg Phe Leu Phe Met Asp Gly
165 170 175

Asn Cys Tyr Tyr Lys Asn Pro Cys Arg Gln Ala Leu Glu Val Ala Pro
180 185 190

Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr

195	200	205
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Leu Leu Leu Ser Tyr Asn Arg Ile Val Lys Leu Ala Pro Glu Asp Leu 225 230 235 240		
Ala Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg 245 250 255		
Arg Cys Asp His Ala Pro Asn Pro Cys Met Glu Cys Pro Arg His Phe 260 265 270		
Pro Gln Leu His Pro Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly 275 280 285		
Leu Val Leu Lys Asp Ser Ser Leu Ser Trp Leu Asn Ala Ser Trp Phe 290 295 300		
Arg Gly Leu Gly Asn Leu Arg Val Leu Asp Leu Ser Glu Asn Phe Leu 305 310 315 320		
Tyr Lys Cys Ile Thr Lys Thr Lys Ala Phe Gln Gly Leu Thr Gln Leu 325 330 335		
Arg Lys Leu Asn Leu Ser Phe Asn Tyr Gln Lys Arg Val Ser Phe Ala 340 345 350		
His Leu Ser Leu Ala Pro Ser Phe Gly Ser Leu Val Ala Leu Lys Glu 355 360 365		
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Leu Thr Ala Thr Met Gly Glu Ala Asp Gly Gly Glu Lys Val Trp Leu
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Gln Pro Gly Asp Leu Ala Pro Ala Pro Val Asp Thr Pro Ser Ser Glu
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Asp Phe Arg Pro Asn Cys Ser Thr Leu Asn Phe Thr Leu Asp Leu Ser
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Arg Asn Asn Leu Val Thr Val Gln Pro Glu Met Phe Ala Gln Leu Ser
 485 490 495

His Leu Gln Cys Leu Arg Leu Ser His Asn Cys Ile Ser Gln Ala Val
 500 505 510

Asn Gly Ser Gln Phe Leu Pro Leu Thr Gly Leu Gln Val Leu Asp Leu
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Ser Arg Asn Lys Leu Asp Leu Tyr His Glu His Ser Phe Thr Glu Leu
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Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Gly
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Met Gln Gly Val Gly His Asn Phe Ser Phe Val Ala His Leu Arg Thr
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Leu Arg His Leu Ser Leu Ala His Asn Asn Ile His Ser Gln Val Ser
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Gln Gln Leu Cys Ser Thr Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn
 595 600 605

Ala Leu Gly His Met Trp Ala Glu Gly Asp Leu Tyr Leu His Phe Phe
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Gln Gly Leu Ser Gly Leu Ile Trp Leu Asp Leu Ser Gln Asn Arg Leu
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His Thr Leu Leu Pro Gln Thr Leu Arg Asn Leu Pro Lys Ser Leu Gln
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Val Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Lys Trp Trp Ser
 660 665 670

Leu His Phe Leu Pro Lys Leu Glu Val Leu Asp Leu Ala Gly Asn Arg
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Leu Lys Ala Leu Thr Asn Gly Ser Leu Pro Ala Gly Thr Arg Leu Arg
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Arg Leu Asp Val Ser Cys Asn Ser Ile Ser Phe Val Ala Pro Gly Phe
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Phe Ser Lys Ala Lys Glu Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala
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Asp Cys Phe Ala Leu Ser Leu Leu Ala Val Ala Leu Gly Leu Gly Val
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Pro Met Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His
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Leu Cys Leu Ala Trp Leu Pro Trp Arg Gly Arg Gln Ser Gly Arg Asp
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Glu Asp Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Thr Gln
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Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Gly Gln Leu Glu
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Glu Cys Arg Gly Arg Trp Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp
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Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser
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Gly Leu Leu Arg Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu
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Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
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Leu Leu Trp Pro His Gln Pro Ser Gly Gln Arg Ser Phe Trp Ala Gln
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<212> PRT

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Leu Pro Cys Glu Leu Lys Pro His Gly Leu Val Asp Cys Asn Trp Leu
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Phe Leu Lys Ser Val Pro Arg Phe Ser Ala Ala Ala Ser Cys Ser Asn
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Ile Thr Arg Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asn
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Ser Asp Phe Val His Leu Ser Asn Leu Arg Gln Leu Asn Leu Lys Trp

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Asn Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Arg Leu Pro Ser Ser	130	135		140	
Leu Val Asn Leu Ser Leu Ser His Thr Asn Ile Leu Val Leu Asp Ala	145	150		155	160
Asn Ser Leu Ala Gly Leu Tyr Ser Leu Arg Val Leu Phe Met Asp Gly	165	170		175	
Asn Cys Tyr Tyr Lys Asn Pro Cys Thr Gly Ala Val Lys Val Thr Pro	180	185		190	
Gly Ala Leu Leu Gly Leu Ser Asn Leu Thr His Leu Ser Leu Lys Tyr	195	200		205	
Asn Asn Leu Thr Lys Val Pro Arg Gln Leu Pro Pro Ser Leu Glu Tyr	210	215		220	
Leu Leu Val Ser Tyr Asn Leu Ile Val Lys Leu Gly Pro Glu Asp Leu	225	230		235	240
Ala Asn Leu Thr Ser Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg	245	250		255	
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Leu His Leu His Pro Glu Thr Phe His His Leu Ser His Leu Glu Gly	275	280		285	
Leu Val Leu Lys Asp Ser Ser Leu His Thr Leu Asn Ser Ser Trp Phe	290	295		300	
Gln Gly Leu Val Asn Leu Ser Val Leu Asp Leu Ser Glu Asn Phe Leu	305	310		315	320

Tyr Glu Ser Ile Asn His Thr Asn Ala Phe Gln Asn Leu Thr Arg Leu
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Arg Lys Leu Asn Leu Ser Phe Asn Tyr Arg Lys Lys Val Ser Phe Ala
 340 345 350

Arg Leu His Leu Ala Ser Ser Phe Lys Asn Leu Val Ser Leu Gln Glu
 355 360 365

Leu Asn Met Asn Gly Ile Phe Phe Arg Ser Leu Asn Lys Tyr Thr Leu
 370 375 380

Arg Trp Leu Ala Asp Leu Pro Lys Leu His Thr Leu His Leu Gln Met
 385 390 395 400

Asn Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Thr Phe Arg Ala
 405 410 415

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Leu Ser Glu Ala Thr Pro Glu Glu Ala Asp Asp Ala Glu Gln Glu Glu
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Leu Leu Ser Ala Asp Pro His Pro Ala Pro Leu Ser Thr Pro Ala Ser
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Lys Asn Phe Met Asp Arg Cys Lys Asn Phe Lys Phe Thr Met Asp Leu
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Ser Arg Asn Asn Leu Val Thr Ile Lys Pro Glu Met Phe Val Asn Leu
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Ser Arg Leu Gln Cys Leu Ser Leu Ser His Asn Ser Ile Ala Gln Ala
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Val Asn Gly Ser Gln Phe Leu Pro Leu Thr Asn Leu Gln Val Leu Asp
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Leu Ser His Asn Lys Leu Asp Leu Tyr His Trp Lys Ser Phe Ser Glu
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Leu Pro Gln Leu Gln Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe
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Ser Met Lys Gly Ile Gly His Asn Phe Ser Phe Val Ala His Leu Ser
565 570 575

Met Leu His Ser Leu Ser Leu Ala His Asn Asp Ile His Thr Arg Val
580 585 590

Ser Ser His Leu Asn Ser Asn Ser Val Arg Phe Leu Asp Phe Ser Gly
595 600 605

Asn Gly Met Gly Arg Met Trp Asp Glu Gly Gly Leu Tyr Leu His Phe
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Phe Gln Gly Leu Ser Gly Leu Leu Lys Leu Asp Leu Ser Gln Asn Asn
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Leu His Ile Leu Arg Pro Gln Asn Leu Asp Asn Leu Pro Lys Ser Leu
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Lys Leu Leu Ser Leu Arg Asp Asn Tyr Leu Ser Phe Phe Asn Trp Thr
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Ser Leu Ser Phe Leu Pro Asn Leu Glu Val Leu Asp Leu Ala Gly Asn
675 680 685

Gln Leu Lys Ala Leu Thr Asn Gly Thr Leu Pro Asn Gly Thr Leu Leu
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Gln Lys Leu Asp Val Ser Ser Asn Ser Ile Val Ser Val Val Pro Ala
705 710 715 720

Phe Phe Ala Leu Ala Val Glu Leu Lys Glu Val Asn Leu Ser His Asn
725 730 735

Ile Leu Lys Thr Val Asp Arg Ser Trp Phe Gly Pro Ile Val Met Asn
740 745 750

Leu Thr Val Leu Asp Val Arg Ser Asn Pro Leu His Cys Ala Cys Gly
755 760 765

Ala Ala Phe Val Asp Leu Leu Leu Glu Val Gln Thr Lys Val Pro Gly
770 775 780

Leu Ala Asn Gly Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg
785 790 795 800

Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Val Leu Ser
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Trp Asp Cys Phe Gly Leu Ser Leu Leu Ala Val Ala Val Gly Met Val
 820 825 830

Val Pro Ile Leu His His Leu Cys Gly Trp Asp Val Trp Tyr Cys Phe
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His Leu Cys Leu Ala Trp Leu Pro Leu Leu Ala Arg Ser Arg Arg Ser
 850 855 860

Ala Gln Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln
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Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Arg Leu Glu
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Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
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